09/660924
AMD#8

=> s hybridiz?
L1 465166 HYBRIDIZ?

=> s "5'"(w)nuclease
L2 580 "5'"(W) NUCLEASE

=> s l1 and l2
L3 105 L1 AND L2

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 56 DUP REM L3 (49 DUPLICATES REMOVED)

=> s l4 and py<1993
1 FILES SEARCHED...
3 FILES SEARCHED...
4 FILES SEARCHED...
L5 1 L4 AND PY<1993

=> d l5 ibib abs 1

L5 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1982:183717 BIOSIS
DOCUMENT NUMBER: BA73:43701
TITLE: STRUCTURE OF THE GENOME OF EQUINE HERPESVIRUS TYPE 1.
AUTHOR(S): HENRY B E; ROBINSON R A; DAUENHAUER S A; ATHERTON S S;
HAYWARD G S; O'CALLAGHAN D J
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. MISSISSIPPI MED. CENT., JACKSON,
MISSISSIPPI 39216.
SOURCE: VIROLOGY, (1981) 115 (1), 97-114.
CODEN: VIRLAX. ISSN: 0042-6822.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The molecular structure of the genome of equine herpesvirus type 1 (EHV-1)
was determined by restriction endonuclease mapping studies. Primary restriction enzyme digestion of purified EHV-1 DNA, either unlabeled, 32PO4 labeled or [3H]TdR [deoxythymidine] labeled, gave the following cleavage patterns: EcoRI yielded 17 fragments of 23.4-1.3 megadaltons (Md); BglII, 16 fragments of 24.5-1.0 Md; XbaI, 15 major fragments of 18.6-1.7 Md; and BamHI, 17 fragments of 13.7-2.8 Md. Several fragments
were present in 0.5 M amounts while all others were 1.0 M; no 0.25 M fragments were detected. Secondary restriction enzyme digestion of these isolated fragments with various enzymes, analysis of terminal fragments using both the methods of .lambda. 5' exonuclease digestion and end labeling with polynucleotide kinase and blot ***hybridization*** experiments with 32P-labeled BamHI fragments indicated that this herpesvirus genome is a 92-Md linear, double-stranded DNA molecule and is
comprised of 2 segments designated L (long) and S (short) which are 71.6 and 20.4 Md, respectively. The 0.5 M fragments are located at the ends of the S region, an arrangement which allows the S region to invert relative to the L region; thus, 2 structural arrangements (isomers) of the genome exist. Areas of heterogeneity were detected at the L terminus, within the S segment and at a split variable locus in the L region.

=> d his

(FILE 'HOME' ENTERED AT 10:10:38 ON 30 OCT 2001)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 10:10:58 ON 30
OCT 2001
L1 465166 S HYBRIDIZ?
L2 580 S "5'"(W)NUCLEASE
L3 105 S L1 AND L2
L4 56 DUP REM L3 (49 DUPLICATES REMOVED)
L5 1 S L4 AND PY<1993

=> s nuclease?
L6 67585 NUCLEASE?

=> s l1 and l6
L7 7531 L1 AND L6

=> s single(w)strand?
L8 110290 SINGLE(W) STRAND?

=> s l1 and l6 and l8
L9 890 L1 AND L6 AND L8

=> s l6(l)l8
L10 5817 L6(L) L8

=> s l1 and l10
L11 750 L1 AND L10

=> s cleav?
L12 436416 CLEAV?

=> s l6(l)l8(l)l12
L13 1363 L6(L) L8(L) L12

=> s l13 and l1
L14 104 L13 AND L1

=> dup rem
ENTER L# LIST OR (END):l14
PROCESSING COMPLETED FOR L14
L15 64 DUP REM L14 (40 DUPLICATES REMOVED)

=> s l15 and py<1994
1 FILES SEARCHED...
2 FILES SEARCHED...
4 FILES SEARCHED...
L16 33 L15 AND PY<1994

=> d l16 ibib abs 1-33

L16 ANSWER 1 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:324385 BIOSIS
DOCUMENT NUMBER: PREV199396032735
TITLE: Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases.
AUTHOR(S): Lyamichev, Victor; Brow, Mary Ann D.; Dahlberg, James E.
(1)
CORPORATE SOURCE: (1) Dep. Biomolecular Chem., University Wisconsin Sch.
Med., 1300 University Ave., Madison, WI 53706 USA
SOURCE: Science (Washington D C), (1993) Vol. 260, No. 5109, pp.
778-783.
ISSN: 0036-8075.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Previously known 5' exonucleases of several eubacterial DNA polymerases
have now been shown to be structure-specific endonucleases that ***cleave*** ***single*** - ***stranded*** DNA or RNA at the bifurcated end of abase-paired duplex. ***Cleavage*** was not coupled
to synthesis, although primers accelerated the rate of ***cleavage*** considerably. The enzyme appeared to gain access to the ***cleavage*** site by moving from the free end of a 5' extension to the bifurcation of the duplex, where ***cleavage*** took place. ***Single*** - ***stranded*** 5' arms up to 200 nucleotides long were ***cleaved***
from such a duplex. Essentially any linear ***single*** - ***stranded*** nucleic acid can be targeted for specific ***cleavage*** by the 5' ***nuclease*** of DNA polymerase through
hybridization with an oligonucleotide that converts the desired ***cleavage*** site into a substrate.

L16 ANSWER 2 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:182660 BIOSIS
DOCUMENT NUMBER: PREV199395093110
TITLE: Nuclease activity of 1,10-phenanthroline-copper: New conjugates with low molecular weight targeting ligands.

AUTHOR(S): Chen, Chi-Hong B.; Mazumder, Abhijt; Constant, Jean-Francois; Sigman, Davis S. (1)
CORPORATE SOURCE: (1) Molecular Biol. Inst., Univ. Calif., Los Angeles, CA 90024
SOURCE: Bioconjugate Chemistry, (1993) Vol. 4, No. 1, pp. 69-77. ISSN: 1043-1802.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The chemical ***nuclease*** activity of 1,10-phenanthroline-copper depends on DNA sequence because the coordination complex has affinity for DNA. In order to target this efficient nucleolytic activity, it is essential to override its inherent specificity. The minimal size of ligands capable of redirecting the specificity has been investigated. A conjugate (HOP) prepared by alkylating Hoechst dye 33258 with 5-(iodoacetamido)-1,10-phenanthroline has a greater preference for A-T rich regions than the unsubstituted 1,10-phenanthroline-copper complex, reflecting the specificity of this A-T-specific minor-groove binder. However, since quaternizing the dye with 5-(iodoacetamido)-1,10-phenanthroline increases its affinity for DNA, the specificity of ***cleavage*** by the conjugate is less than the binding selectivity of the dye. Linking 1,10-phenanthroline with the peptide of the helix-turn-helix domain of the Trp repressor specificity results in a conjugate with greater reactivity for the operator sequence than the unsubstituted complex. The intrinsic affinity of the 1,10-phenanthroline-Cu can only be partially overriden b the conformationally unstable peptide. Attachment of 1,10-phenanthroline to a deoxyoligonucleotide complementary to a ***single*** - ***stranded*** loop of RNA successfully targets the scission of the chemical ***nuclease*** . ***Cleavage*** sites are observed not only contiguous to the site of ***hybridization*** but also at nonadjacent sequence positions. The latter set of sites must be close in space to the 5' end of the ***hybridized*** deoxyoligonucleotide.

L16 ANSWER 3 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:364443 BIOSIS
DOCUMENT NUMBER: BA92:52668
TITLE: SINGLE-STRANDED REPLICATION INTERMEDIATES OF RIBOSOMAL DNA REPLICONS OF PEA.
AUTHOR(S): VAN'T HOF J; LAMM S S
CORPORATE SOURCE: BIOLOGY DEP., BROOKHAVEN NATL. LAB., UPTON, N.Y. 11973.
SOURCE: EMBO (EUR MOL BIOL ORGAN) J, (1991) 10 (7), 1949-1954.
CODEN: EMJODG. ISSN: 0261-4189.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Replication of ribosomal DNA replicons in cells of Pisum sativum (cv. Alaska) occurs bidirectionally by displacement loops. Replication is initiated on opposite parental strands and nascent chains are elongated moving 5' .fwdarw. 3' along each parental template. Replicative intermediates were analyzed by 2-dimensional agarose gel electrophoresis under neutral - neutral and neutral - alkaline conditions. Southern blots of ribosomal DNA fragments separated in the second dimension under neutral conditions show slowly migrating replicative fragments that ***hybridize*** with specific probes in a manner consistent with bidirectional replication. The replicative fragments are present in root meristems with cells in G2 phase. The following observations indicate that the replicative fragments are ***single*** ***stranded*** . The apparent length of the replicative fragments is not the same when separated under neutral and alkaline conditions. They contain rDNA without breaks and they do not exhibit the smaller nascent chains expected from replication bubbles and forks. They are not ***cleaved*** by restriction enzymes that require duplex DNA as substrate and they are digestible by S1 ***nuclease*** .

L16 ANSWER 4 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:110115 BIOSIS
DOCUMENT NUMBER: BA91:57505
TITLE: PHYLOGENETIC ANALYSIS AND SECONDARY STRUCTURE OF THE BACILLUS-SUBTILIS BACTERIOPHAGE RNA REQUIRED FOR DNA PACKAGING.
AUTHOR(S): BAILEY S; WICHITWECHKARN J; JOHNSON D; REILLY B E; ANDERSON D L; BODLEY J W
CORPORATE SOURCE: DEP. BIOCHEM., UNIV. MINN., MINNEAPOLIS, MINN. 55455.
SOURCE: J BIOL CHEM, (1990) 265 (36), 22365-22370.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An unusual RNA molecule encoded by the Bacillus subtilis bacteriophage .vphi.29 is a structural component of the viral prohead and is required for the ATP-dependent packaging of DNA. Here we report a model of secondary structure for this prohead RNA developed from a phylogenetic analysis of the primary sequences of prohead RNAs of related phages. Twenty-nine phages related to .vphi.29 were found to produce prohead RNAs. These RNAs were analyzed by their ability to replace .vphi.29 RNA in vitro phage assembly, by Northern blot ***hybridization*** with a probe complementary to .vphi. RNA, and by partial and complete sequence analyses. These analyses revealed four quite different sequences ranging in length from 161 to 174 residues. The secondary structure deduced from these sequences, in agreement with earlier observations, indicated that prohead RNA is organized into two domains. The larger 5'-domain (Domain I) is composed of 113-117 residues and contains four helices. Three of these helices appear to be organized into a central stem that is interrupted by two unpaired loops and the fourth helix and loop. The smaller 3'-domain (Domain II) is composed of 40-44 residues and consists of two helices. Domains I and II are separated by 8-13 unpaired residues. Nuclease cleavage occurs ***readily*** ***in*** this single-stranded joining ***region*** , ***and*** this cleavage allows the ***subsequent*** separation of the two RNA domains. The separated Domain I is fully active in DNA packaging in vitro. The functional significance and biological role of Domain II are unknown. The phylogenetic secondary structure model provides a basis for further analysis of the role of this RNA in bacteriophage morphogenesis.

L16 ANSWER 5 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1987:23121 BIOSIS
DOCUMENT NUMBER: BA83:13055
TITLE: NUCLEASE ACTIVITY OF 1 10 PHENANTHROLINE-COPPER SEQUENCE-SPECIFIC TARGETING.
AUTHOR(S): CHEN C-H B; SIGMAN D S
CORPORATE SOURCE: DEP. BIOLOGICAL CHEMISTRY, SCH. MED., MOLECULAR BIOLOGY INST., UNIV. CALIFORNIA, LOS ANGELES, CA 90024.
SOURCE: PROC NATL ACAD SCI U S A, (1986) 83 (19), 7147-7151.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The ***nuclease*** activity of 1,10-phenanthroline-copper ion can be targeted to specific DNA sequences by attachment of the ligand to the 5' end of complementary deoxyoligonucleotides via a phosphoramidate linkage. To synthesize the adduct, the phosphorimidazolide of the deoxyoligonucleotide is prepared using a water-soluble carbodiimide and is then coupled to 5-glycylamido-1,10-phenanthroline. After ***hybridization*** to the target DNA, sequence-specific ***cleavage*** is observed upon the addition of cupric ion and 3-mercaptopropionic acid. Two methods of assaying the cutting of the operator sequence of the lac operon have been employed using the oligonucleotide 5'-AATTGTTATCCGCTCACAATT-3' representing sequence positions 21-1 of the template strand. In the first, the ***single*** - ***stranded*** DNA of the phage M13mp8 was the target, and cuts were detected using a primer-extension assay. In the second, the substrate was an EcoRI fragment 3' labelled in the nontemplate strand. After denaturation and reannealing to the oligonucleotide-1,10-phenanthroline adduct, cupric ion and 3-mercaptopropionic acid were added, and the

products were analyzed directly on a sequencing gel. With the phenanthroline moiety attached to position 21 of the oligonucleotide carrier, cutting was observed at positions 20-25 using both assays.

L16 ANSWER 6 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1986:122855 BIOSIS
DOCUMENT NUMBER: BA81:33271
TITLE: DOUBLE-STRAND CLEAVAGE AT A TWO-BASE DELETION MISMATCH IN A DNA HETERODUPLEX BY NUCLEASE S-1.
AUTHOR(S): BURDON M G; LEES J H
CORPORATE SOURCE: DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY, UNIVERSITY OF ST. ANDREWS, IRVINE BUILDING, NORTH STREET, ST. ANDREWS, FIFE KY16 9AL.
SOURCE: BIOSCI REP, (1985) 5 (8), 627-632. CODEN: BRPTDT. ISSN: 0144-8463.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A two-base deletion mismatch was generated in a DNA heteroduplex by ***hybridization*** of two linear plasmid DNA molecules differing only by the presence of a two-base deletion in one of them. The heteroduplex was shown to be sensitive to double-strand ***cleavage*** by ***nuclease*** S1, thus demonstrating the potential value of ***single*** - ***stranded*** probes for the detection of polymorphisms in genomic DNA due to very small deletions.

L16 ANSWER 7 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1984:266422 BIOSIS
DOCUMENT NUMBER: BA78:2902
TITLE: S-1 NUCLEASE MAPPING ANALYSIS OF RIBOSOMAL RNA PROCESSING IN WILD TYPE AND PROCESSING DEFICIENT ESCHERICHIA-COLI.
AUTHOR(S): KING T C; SCHLESSINGER D
CORPORATE SOURCE: DIV. BIOL. BIOMED. SCI., WASH. UNIV. SCH. MED., ST. LOUIS, MO 63110.
SOURCE: J BIOL CHEM, (1983) 258 (19), 12034-12042. CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB S1 ***nuclease*** mapping was used to assess rRNA processing in E. coli. ***Single*** - ***stranded*** DNA probes complementary to the sequences bordering each terminus of 16 and 23 S rRNA were end-labeled, ***hybridized*** to total E. coli RNA and treated with S1 ***nuclease*** . The resultant DNA fragments were then displayed on denaturing polyacrylamide gels. Measurements of steady state levels of precursor rRNA species and measurements of the rates of decay of precursors after transcription arrest by rifampicin gave consistent results. The rRNA precursor species identified in wild type cells corresponded to those previously identfied by other means. In RNase III-deficient strains, mature 16 S rRNA termini form at the same rate as in wild type cells; but the normal mature termini of 23 S rRNA are never generated. RNase III ***cleavage*** at the 5' end of 23 S rRNA can occur before the 3' end of the same molecule is synthesized. The ***cleavages*** that generate the mature termini of 16 S rRNA are interdependent; in the BUMMER strain, slow processing at the 5' end is accompanied by slow processing at the 3' end. The kinetically observed order of processing reactions is obligate for some ***cleavages*** but not for others and the assumption that complete rRNA processing is required for function fails for 23 S rRNA.

L16 ANSWER 8 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1983:160383 BIOSIS
DOCUMENT NUMBER: BA75:10383
TITLE: STRATEGIES FOR CONSTRUCTING COMPLEMENTARY DNA FOR CLONING.
AUTHOR(S): GAUBATZ J; PADDOCK G V
CORPORATE SOURCE: DEP. BIOCHEM., MSB 2170, COLL. MED., UNIV. SOUTH ALABAMA, MOBILE, ALABAMA 36688, U.S.A.
SOURCE: J THEOR BIOL, (1982) 95 (4), 679-696. CODEN: JTBIAP. ISSN: 0022-5193.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Alternative approaches to existing methods were examined for synthesizing complementary DNA suitable for molecular cloning. One model of construction is presented in which ribonucleotides are added to the 3' end of complementary DNA prior to synthesis of the 2nd DNA strand. The hairpin structure at one end of the molecule is then opened by treatment with RNase or alkali. This method would eliminate the normal requirement for ***single*** - ***strand*** specific ***nucleases*** and thus shows promise as a means for preserving the 5' end sequences of mRNA in recombinant complementary DNA studies. Another technique for constructing complementary DNA is proposed in which no ***cleavage*** step is required. A hairpin, double-stranded DNA is extended with a homopolymer at the 3' end, and displacement or 3rd-strand synthesis by the Klenow fragment of DNA polymerase I is primed by an oligonucleotide ***hybridized*** to the homopolymer. The end result should be an inverted repeat with 2-fold rotational symmetry. The mRNA 5' end sequences represent the center of symmetry. Cloned, symmetrical DNA should facilitate subsequent nucleotide sequence analysis. The symmetrical molecule may serve as an intermediate in continued DNA synthesis provided the homopolymer chain is sufficiently longer than the primer, thus leading to mRNA sequence amplification in vitro. Alternative options with attendant advantages and disadvantages are given at each stage in the construction schemes. These strategies, along with established procedures, offer a repertoire from which researchers may select in order to fill their specific needs.

L16 ANSWER 9 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1979:274985 BIOSIS
DOCUMENT NUMBER: BA68:77489
TITLE: IN-VITRO SYNTHESIS AND CHARACTERIZATION OF DNA COMPLEMENTARY TO CADANG-CADANG DISEASE ASSOCIATED RNA.
AUTHOR(S): RANDLES J W; PALUKAITIS P
CORPORATE SOURCE: DEP. PLANT PATHOL., WAITE AGRIC. RES. INST., UNIV. ADELAIDE, ADELAIDE, S. AUST., AUST.
SOURCE: J GEN VIROL, (1979) 43, 649-662. CODEN: JGVIAY. ISSN: 0022-1317.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The anomalous viroid-like RNA associated with cadang-cadang disease of coconut palms (ccRNA-1) was ***cleaved*** by treatment with the ***single*** - ***strand*** specific ***nuclease*** S1, polyadenylated and used as a template for the oligo(dT) primed synthesis of complementary (c)DNA by the avian myeloblastosis virus reverse transcriptase. The efficiency of synthesis was low, with only 3-4.5 ng of cDNA synthesized from 2 .mu.g of RNA. Most of the cDNA was in the 4S size class. A R0t1/2 [R0 = initial concentration, t1/2 = half ***hybridization*** time] value of 1 .times. 10-3 mol s/l was obtained when this cDNA was ***hybridized*** with ccRNA-1, consistent with ccRNA-1 representing a unique species of MW about 100,000. The maximum ***hybridization*** value obtained with ccRNA-1 was about 50%; the S1 ***nuclease*** resistance of the cDNA after self-annealing was about 7%. The melting behavior of the homologous hybrids provided evidence for the specificity of base-pairing with no evidence of mismatching. The cDNA was a specific probe for cadang-cadang associated RNA. It was used to demonstrate that ccRNA-1 and ccRNA-2 have common nucleotide sequences, that ccRNA-1 is uniquely associated with diseased and not healthy palms and that it has no significant homology with high MW RNA or DNA from diseased palms. The value of the cDNA as a diagnostic probe for ccRNA-1 in

crude nucleic acid extracts was demonstrated.

L16 ANSWER 10 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1978:245843 BIOSIS
DOCUMENT NUMBER: BA66:58340
TITLE: THE TRANSCRIPTION MAP OF MOUSE MITOCHONDRIAL DNA.
AUTHOR(S): BATTEY J; CLAYTON D A
CORPORATE SOURCE: LAB. EXP. ONCOL., DEP. PATHOL., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
SOURCE: CELL, (1978) 14 (1), 143-156. CODEN: CELLB5. ISSN: 0092-8674.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Nine transcripts complementary to mouse fibroblast L cell mitochondrial DNA were detected, sized and mapped to restriction fragments using the method of Berk and Sharp. RNA isolated from L cell mitochondria was ***hybridized*** to 32P-labeled, cloned L cell mitochondrial DNA restriction fragments in 70% foramide under conditions 5.degree. C above the melting temperature of the DNA-DNA duplex, but approximately 15.degree. C below the melting temperature of the RNA-DNA duplex. The heteroduplexed material was then treated with the ***single*** - ***strand*** -specific ***nuclease*** S1, which ***cleaves*** the ***single*** - ***stranded*** DNA not protected by RNA-DNA duplex formation into oligonucleotides and leaves intact 32P-labeled, ***single*** - ***stranded*** DNA replicas complementary to the transcripts. The ***single*** - ***stranded*** DNA replicas were then resolved and sized by alkaline agarose gel electrophoresis. ***Hybridization*** to strand-separated, 32P-labeled L cell mitochondrial DNA restriction fragments under the same conditions showed that all 9 transcripts ***hybridized*** exclusively to the heavy strand (H strand) of restriction fragments isolated as the dense strand from alkaline CsCl gradients, indicating that all stable transcripts 300 bases or longer detected by this technique originate from genes on the H strand. The 2 most abundant transcripts homologous to mitochondrial DNA map adjacent to the origin of replication. This is consistent with map positions assigned to the large and small mitochondrial ribosomal RNA isolated from Xenopus laevis and HeLa [human cervical cancer] cells. Six of the other 7 transcripts map continuously in approximately 40% of the genome. Only 1 transcript of 950 bases maps in the 1st guadrant of the genome as defined by the origin and direction of mitochondrial DNA replication, and it does not lie within the D loop region. The genetic function of the remaining 75% of this region of the genome is yet to be determined.

L16 ANSWER 11 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1977:148604 BIOSIS
DOCUMENT NUMBER: BA63:43468
TITLE: ISOLATION AND SOME PROPERTIES OF A MAMMALIAN RIBOSOMAL DNA.
AUTHOR(S): BLIN N; STEPHENSON E C; STAFFORD D W
SOURCE: CHROMOSOMA (BERL), (1976) 58 (1), 41-50. CODEN: CHROAU. ISSN: 0009-5915.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
AB The DNA coding for 28 S and 18 S rRNA, including the spacer regions, was isolated from calf (Bos taurus) thymus gland. The method used included shearing of the total DNA to a highly homogeneous size population, selective heat denaturation and S 1 ***nuclease*** treatment to remove ***single*** ***stranded*** DNA. Repeated centrifugation on density gradients yields a 140-fold purified rDNA fraction with a GC [guanine cytosine] content of 61.2%. Eco-R-I ***nuclease*** ***cleaves*** this DNA into 2 fragments of 16.4 and 4.9 .times. 106 daltons. ***Hybridization*** of these fragments with 28 S and 18 S rRNA shows that the 28 S coding sequence is located mostly on the 4.9 .times. 106 dalton fragment, while both the 16.4 and 4.9 .times. 106 dalton fragments contain the 18 S sequence. The data indicate that the rRNA gene has a repeat unit of 21.3 .times. 106 daltons which includes a nontranscribed spacer of about 12.5 .times. 106 daltons.

L16 ANSWER 12 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1977:106099 BIOSIS
DOCUMENT NUMBER: BA63:963
TITLE: FREE RIBOSOMAL DNA MOLECULES FROM TETRAHYMENA-PYRIFORMIS GL ARE GIANT PALINDROMES.
AUTHOR(S): ENGBERG J; ANDERSSON P; LEICK V; COLLINS J
SOURCE: J MOL BIOL, (1976) 104 (2), 455-470. CODEN: JMOBAK. ISSN: 0022-2836.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
AB Restriction endonuclease EcoRI was used to study the structure of the free ribosomal DNA molecules from Tetrahymena pyriformis, strain GL. It was concluded that the free DNA molecules from Tetrahymena are giant palindromes, each containing 2 genes for preribosomal RNA arranged in rotational symmetry as inverted repeating sequences. Analyses of the sizes of products of partial or complete digestion and quantitative analyses of the products of complete digestion of uniformly 32P-labeled rDNA yielded an RI endonucleolytic ***cleavage*** map which showed that the EcoRI recognition sites are arranged symmetrically about the center of the rDNA molecule. When heat-denatured rDNA was rapidly cooled under conditions in which no renaturation would occur between separated complementary strands of DNA, molecules of half the size of the original rDNA molecule were produced. These were double-stranded DNA molecules as evidenced by their resistance to digestion with S1 ***nuclease*** . Moreover, they could be digested with EcoRI to produce fragments of sizes which would be predicted from the assumption that each ***single*** ***strand*** of the original rDNA molecule had folded back on itself to form a 'hair-pin" double-stranded DNA structure. ***Hybridization*** experiments between ribosomal RNA and purified rDNA showed that each rDNA molecule contains 2 genes for rDNA. ***Hybridization*** of the isolated EcoRI fragments of rDNA with 25 S or 17 S rRNA suggested that the 2 structural genes for 17 S rRNA are located near the center of the rDNA molecule and the 2 genes for 25 S rRNA are found in distal positions.

L16 ANSWER 13 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 76046501 EMBASE
DOCUMENT NUMBER: 1976046501
TITLE: Studies on reverse transcriptase of RNA tumor viruses. III. Properties of purified Moloney murine leukemia virus DNA polymerase and associated RNase H.
AUTHOR: Verma I.M.
CORPORATE SOURCE: Tum. Virol. Lab., Salk Inst., San Diego, Calif. 92112, United States
SOURCE: Journal of Virology, (1975) 15/4 (843-854). CODEN: JOVIAM
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
016 Cancer
025 Hematology
023 Nuclear Medicine
LANGUAGE: English
AB DNA polymerase was purified from a cloned isolate of Moloney murine leukemia virus (M MuLV). Purified M MuLV DNA polymerase, upon analysis by polyacrylamide gel electrophoresis, showed one major polypeptide of mol wt 80,000. Estimation of molecular weight from the sedimentation rate of the purified enzyme in a glycerol gradient was consistent with a structure containing one polypeptide. M MuLV DNA polymerase could transcribe ribopolymers, deoxyribopolymers, and heteropolymers as efficiently as did purified DNA polymerase from avian myeloblastosis virus (AMV). M MuLV DNA polymerase, however, transcribed native 70S viral RNA less efficiently than did AMV DNA polymerase. Addition of oligo(dT) enhanced 5 to 10 fold

the transcription of 70S viral RNA by M MuLV DNA polymerase. Purified enzyme also exhibited ***nuclease*** activity (RNase H) that selectively degraded the RNA moiety of the RNA DNA hybrid. It did not degrade ***single*** ***stranded*** RNA, ***single*** ***stranded*** DNA, double stranded RNA, and double stranded DNA. M MuLV DNA polymerase associated RNase H acted as a random exonuclease. When [3H]poly(A)-poly(dT) was used as a substrate, the size of the M MuLV DNA polymerase associated RNase H digested product was larger than the size of the digestion products by AMV DNA polymerase. The oligonucleotide digestion products could be further digested to 5' AMP by snake venom phosphodiesterase, indicating that the products were terminated by 3 6 oh groups. Alkaline hydrolysis of the oligonucleotide digestion products generated pAp, suggesting that M MuLV DNA polymerase associated RNase H ***cleaves*** at the 3' side of the 3',5' phosphodiester bond. The ratios of the rates of DNA polymerase activity and RNase H activity were not significantly different in the murine and avian enzymes.

L16 ANSWER 14 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 75124609 EMBASE
DOCUMENT NUMBER: 1975124609
TITLE: Ribonucleotides in newly synthesized DNA of herpes simplex virus.
AUTHOR: Biswal N.; Murray B.K.; Benyesh Melnick M.
CORPORATE SOURCE: Dept. Virol., Epidemiol., Baylor Coll. Med., Houston, Tex. 77025, United States
SOURCE: Virology, (1974) 61/1 (87-99).
CODEN: VIRLAX
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
047 Virology
013 Dermatology and Venereology
LANGUAGE: English
AB Newly synthesized DNA of herpes simplex virus type 1 (HSV-1), obtained from primary rabbit kidney cells pulse labeled with (3H) thymidine or (3H) uridine at 6 hr postinfection, was purified by two cycles of centrifugation in CsCl density gradients. These intracellular viral DNA preparations ***hybridized*** specifically with homologous HSV-1 DNA but not with host cell DNA or E. coli DNA. Upon denaturation by alkali, the (3H) thymidine labeled HSV 1 DNA ***cleaved*** to smaller pieces. The alkali labile material in the viral DNA was identified as ribonucleotides on the basis of the following observations: (1) When labeled with (3H) uridine for short periods, the labeled virus 'DNA' was susceptible to RNase and NaOH, and all the radioactivity was confined to the nucleoside (3H) uridine; however, upon longer labeling periods (up to 20 hr), the (3H) uridine labeled viral 'DNA' became more susceptible to DNase, as most but not all of the (3H) uridine was converted to deoxyribonucleosides. (2) Denaturation of (3H) uridine labeled double stranded HSV 1 'DNA'(p(Cs2SO4) = 1.45 g/cm3) by heat shifted the buoyant density to ***single*** ***stranded*** DNA region (P(Cs2SO4) = 1.48-1.50 g/cm3) but not to ***single*** ***stranded*** RNA region; however, treatment with hot NaOH considerably reduced the radioactivity of this 'DNA'. Treatment with DNase, but not with pronase, shifted the buoyant density to the heavier RNA region of the gradient. Heat denatured DNA but not the native DNA was susceptible to ***single*** ***strand*** specific ***nuclease*** S1.

L16 ANSWER 15 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 75095440 EMBASE
DOCUMENT NUMBER: 1975095440
TITLE: Characterization of a rearrangement in viral DNA: mapping of the circular simian virus 40 like DNA containing a triplication of a specific one third of the viral genome.
AUTHOR: Khoury G.; Fareed G.C.; Berry K.; et al.
CORPORATE SOURCE: Lab. Biol. Viruses, Nat. Inst. Allergy Infect. Dis., NIH, Bethesda, Md. 20014, United States
SOURCE: Journal of Molecular Biology, (1974) 87/2 (289-301).
CODEN: JMOBAK
DOCUMENT TYPE: Journal
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
047 Virology
LANGUAGE: English
AB Serial passage of the non defective form of a simian virus 40 like virus (DAR) isolated from human brain results in the appearance of 3 distinct classes of supercoiled DNAs: R(I) resistant, R(I) sensitive (1 ***cleavage*** site) and R(I) 'supersensitive' (3 ***cleavage*** sites). The R(I) ***cleavage*** product of the 'supersensitive' form is 1/3 the physical size of simian virus 40 DNA (10.4 S) and reassociates about 3 times more rapidly than 'standard' viral DNA. To identify the portions of the DAR genome present in the 10.4 S segment, the plus strand of each of the 11 fragments of 32P labeled simian virus 40 DNA, produced by ***cleavage*** with the Hemophilus influenzae restriction endonuclease, was ***hybridized*** in solution with the sheared R(I) ***cleavage*** product of the 'supersensitive' class of viral DNA. Reaction was observed with fragments located in 2 distinct regions of the simian virus 40 genome: Hin A and C; Hin G, J, F and K. Further studies indicated that simian virus 40 complementary RNA transcribed in vitro with Escherichia coli RNA polymerase from one strand of simian virus 40 DNA reacts with both strands of the denatured 10.4 S ***cleavage*** product when ***hybridization*** is monitored with hydroxyapatite. Treatment of the 10.4 S DNA simian virus 40 cRNA hybrid with the ***single*** ***strand*** specific ***nuclease*** , S1, converted approximately 50% of the radioactive counts to an acid soluble product. These results indicate that the 10.4 S product contains a transposition of sequences originally present on one of the DAR DNA strands to the other strand. Examination of heteroduplexes formed between the 10.4 S segment and unique linear forms of DAR DNA produced with the R.Eco RI restriction endonuclease have confirmed these observations. Thus it appears that a molecular rearrangement(s) has resulted in the recombination and inversion of viral DNA sequences from two separate loci on the parental DAR genome. This 1.1x106 dalton segment is reiterated 3 times in a supercoiled molecule equivalent in physical size to parental DAR DNA.

L16 ANSWER 16 OF 33 MEDLINE
ACCESSION NUMBER: 92366508 MEDLINE
DOCUMENT NUMBER: 92366508 PubMed ID: 1502170
TITLE: Torsional stress generated by RecA protein during DNA strand exchange separates strands of a heterologous insert.
COMMENT: Erratum in: Proc Natl Acad Sci U S A 1992 Dec 15;89(24):12210
AUTHOR: Jwang B; Radding C M
CORPORATE SOURCE: Department of Genetics, Yale University School of Medicine, New Haven, CT 06510.
CONTRACT NUMBER: GM33504 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, ***(1992 Aug 15)*** 89 (16) 7596-600.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920925
Last Updated on STN: 19970203
Entered Medline: 19920915
AB Previous studies have shown that the helical RecA nucleoprotein filament formed on a circular ***single*** ***strand*** of DNA causes the

progressive, directional transfer of a complementary strand from naked linear duplex DNA to the nucleoprotein filament, even when the duplex contains a sizable heterologous insertion. Since RecA protein lacks demonstrable helicase activity, the mechanism by which it pushes strand exchange through long heterologous inserts has been a quandary. In the present study, a linear duplex substrate with an insertion of 110 base pairs in its middle yielded the expected products, whereas much less of the heteroduplex product was seen when the insertion was located at either end of the duplex substrate or 160 base pairs from the far end of the duplex substrate. In an ongoing reaction of the substrate with an insertion in its middle, P1 ***nuclease*** ***cleaved*** intermediates from the point of the insertion to various distal sites. Acting on a duplex substrate that contained a single nick located in the complementary strand just beyond the insertion, RecA protein formed joint molecules but failed to complete strand exchange. These data show that negative torsional stress is generated by distant homologous interactions that occur beyond the heterologous insertion and that such stress is essential for unwinding a heterologous insertion that otherwise halts strand exchange.

L16 ANSWER 17 OF 33 MEDLINE
ACCESSION NUMBER: 89255284 MEDLINE
DOCUMENT NUMBER: 89255284 PubMed ID: 2542276
TITLE: Predicted structures of apolipoprotein II mRNA constrained by nuclease and dimethyl sulfate reactivity: stable secondary structures occur predominantly in local domains via intraexonic base pairing.
AUTHOR: Hwang S P; Eisenberg M; Binder R; Shelness G S; Williams D L
CORPORATE SOURCE: Department of Pharmacological Sciences, State University of New York, Stony Brook 11794.
CONTRACT NUMBER: DK18171 (NIDDK)
GM 07518 (NIGMS)
GM 08065 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, ***(1989 May 15)*** 264 (14) 8410-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198906
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19970203
Entered Medline: 19890630
AB Analyses of apolipoprotein II mRNA with chemical and enzymatic probes showed that double- and ***single*** - ***stranded*** regions were distributed uniformly along the mRNA except for a large (72 nucleotides) ***single*** - ***stranded*** region containing the translation stop codon. Secondary structure models constrained by the experimental data were made by varying the distance (along the mRNA) over which base pairing was allowed. Four prominent secondary structures were seen with restrictions of 165, 330, or 659 nucleotides suggesting that such structures from via local interactions over distances of 50-120 nucleotides. Predicted long range interactions involve only 2-3 base pairs while local interactions involve helices of 4-10 base pairs. Predicted helices of greater than or equal to 4 base pairs occur primarily within exons, raising the possibility that prominent secondary structures in mRNAs may be largely due to intraexonic base pairing. Tests of single- and double-stranded domains by oligonucleotide-directed RNase H ***cleavage*** and primer extension were in accord with the structure model and with ***nuclease*** and chemical modification data. The model predicting base pairing between the coding and the 3' noncoding regions was tested by RNase H ***cleavage*** followed by oligo(dT)-cellulose chromatography to separate 5' and 3' mRNA fragments. Most (82%) of the 5' fragment remained associated with the 3' noncoding region in a structure with a tm = 50 degrees C in 0.2 M Na+ suggesting that this stem could be stable in vivo. This stem may be stable in the isolated mRNA, but would likely occur transiently in polyribosomal apolipoprotein II mRNA due to ribosome transit through the 5' side of the stem. Alternate structures may occur in this region during ribosome transit and play a role in translation termination or in determining the susceptibility of the mRNA to degradation.

L16 ANSWER 18 OF 33 MEDLINE
ACCESSION NUMBER: 89066749 MEDLINE
DOCUMENT NUMBER: 89066749 PubMed ID: 3198630
TITLE: Ribosomal RNA processing. Limited cleavages of mouse preribosomal RNA by a nucleolar endoribonuclease include the early +650 processing site.
AUTHOR: Shumard C M; Eichler D C
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa 33612.
CONTRACT NUMBER: GM29162 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, ***(1988 Dec 25)*** 263 (36) 19346-52.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198901
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890124
AB A highly purified nucleolar associated endoribonuclease was tested for possible involvement in the processing of precursor ribosomal RNA at a primary ***cleavage*** site approximately 650 nucleotides downstream from the transcription initiation site. Preribosomal RNA sequences containing the +650 region were synthesized in vitro and subsequently digested over a range of concentrations of the nucleolar endoribonuclease. ***Cleavages*** generated by the nucleolar endoribonuclease were localized both by S1 ***nuclease*** protection analysis and primer extension analysis. A more precise determination of the specificity of ***cleavage*** was achieved by chemical ***cleavage*** DNA sequence analysis. These data demonstrated that the purified nucleolar endoribonuclease specifically ***cleaved*** the precursor ribosomal RNA transcript at the +650 site. Additional enzyme-dependent ***cleavages*** were observed upstream to the +650 site in a region which is rapidly degraded following processing at the +650 site in vivo. No major ***cleavages*** were observed for a distance of approximately 250 nucleotides downstream from the +650 site in a conserved region of sequence previously shown to be important in specifying processing at the +650 site. As a control, pancreatic ribonuclease, a ***single*** ***strand*** -specific endoribonuclease, was shown not to produce similar ***cleavages*** in the +650 region, indicating that ***cleavage*** by the nucleolar RNase was not simply due to accessibility of the RNA at the +650 site. Taken together, these results suggest that the nucleolar endoribonuclease may be necessary and sufficient to catalyze one of the initial endonucleolytic ***cleavages*** in preribosomal RNA processing.

L16 ANSWER 19 OF 33 MEDLINE
ACCESSION NUMBER: 87169721 MEDLINE
DOCUMENT NUMBER: 87169721 PubMed ID: 2435917
TITLE: Specific endonucleolytic cleavage sites for decay of Escherichia coli mRNA.
AUTHOR: Cannistraro V J; Subbarao M N; Kennell D
CONTRACT NUMBER: GM34127 (NIGMS)
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, ***(1986 Nov 20)*** 192 (2) 257-74.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198705
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19970203

Entered Medline: 19870506

AB The polycistronic lac mRNA of Escherichia coli contains three messages.
The rate of degradation of the second (lacY) message was observed to be equal to that of the third (lacA), and each decayed twice as fast as did the first (lacZ). Specific 5'- and 3'-ended lacY mRNA molecules could be recovered from cells; most likely, they are generated from endonucleolytic ***cleavages*** that are a part of the degradative process. They were observed by S1 ***nuclease*** mapping, and the exact 5'- and 3'-end oligonucleotides of many of them were identified by direct sequencing. Almost all of the molecules started with a 5' adenosine that would be preceded by a pyrimidine. The specificity was further restricted by neighboring nucleotides, and analysis of the data suggested that 5'-U-U decreases-A-U- is especially vulnerable. Also, computer analyses predicted
the most stable secondary structures of selected segments of the mRNA and
suggested that ***cleavages*** may only occur in regions of ***single*** ***strandedness*** . A model of mRNA degradation is proposed based on these observations and earlier ones. There is no unique target on a message for the initial inactivating attack: any region free of ribosomes is vulnerable, but for statistical reasons the initial attack of most molecules is near the ribosome-loading site. With no further ribosome loading, the newly unprotected 5' ends are "chopped off" at one of the next preferred target sites almost as fast as the last ribosomes moves down the mRNA.

L16 ANSWER 20 OF 33 MEDLINE
ACCESSION NUMBER: 87089776 MEDLINE
DOCUMENT NUMBER: 87089776 PubMed ID: 3025644
TITLE: Expression of a human cytomegalovirus late gene is posttranscriptionally regulated by a 3'-end-processing event occurring exclusively late after infection.
AUTHOR: Goins W F; Stinski M F
CONTRACT NUMBER: AI-13562 (NIAID)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, ***(1986 Dec)*** 6 (12)
4202-13.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M14677
ENTRY MONTH: 198702
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19870213

AB A phenomenon of posttranscriptional regulation has been previously identified in cytomegalovirus-infected human fibroblast cells (Wathen and Stinski, J. Virol. 41:462, 1982). A region typifying this phenomenon has been located within the large unique component of the viral genome (map units 0.408 to 0.423). Even though this transcriptional unit was highly transcribed at early times after infection, mRNAs from this region were only detectable on the polyribosomes after viral DNA replication. Thus, this region is believed to code for a late gene. ***Single*** - ***strand*** -specific ***nuclease*** mapping experiments of viral transcripts established that the transcriptional initiation sites and the 5' ends of a downstream exon were identical at early and late times. However, the late transcripts differed from the early transcripts by the processing of the 3' end of the viral RNAs. This involved either the removal of a distinct region of the transcript by the selection of an upstream ***cleavage*** and polyadenylation site or the differential splicing of the RNA molecule. The upstream ***cleavage*** and polyadenylation site was identified by ***nuclease*** mapping analyses
and DNA sequencing. The 3'-end processing of these transcripts is necessary for the detection of these viral RNAs within the cytoplasm of the infected cell. We propose that human cytomegalovirus either codes for a factor(s) that is involved in the 3'-end-processing event at late times after infection or stimulates the synthesis of a host cell factor(s) involved in this complex regulatory event. This level of regulation may have an influence on the types of cells that permit productive cytomegalovirus replication.

L16 ANSWER 21 OF 33 MEDLINE
ACCESSION NUMBER: 85269599 MEDLINE
DOCUMENT NUMBER: 85269599 PubMed ID: 2991843
TITLE: S1-sensitive sites in the supercoiled double-stranded form of tomato golden mosaic virus DNA component B: identification of regions of potential alternative secondary structure and regulatory function.
AUTHOR: Sunter G; Buck K W; Coutts R H
SOURCE: NUCLEIC ACIDS RESEARCH, ***(1985 Jul 11)*** 13 (13)
4645-59.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198509
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850904

AB The sensitivity of the supercoiled double-stranded form of the DNA of tomato golden mosaic virus (TGMV), a geminivirus, to the ***single*** - ***strand*** specific enzyme S1 ***nuclease*** has been demonstrated. Specific S1 ***cleavage*** sites were identified in TGMV
DNA component B by cloning into the ***single*** - ***strand*** bacteriophage vector M13 mp8 and sequencing of the inserted DNA. Analysis
of the DNA sequence at the sites of S1 sensitivity in TGMV DNA component B
revealed several possible regions of alternative secondary structure which were clustered in an intergenic region upstream of the starts of the two major open reading frames which are in opposite orientations. This region contains putative transcriptional promoter and modulatory sequences and a possible replication origin. The extreme S1 sensitivity of the supercoiled form of TGMV DNA component A precluded its cloning under the conditions
employed for selective ***cleavage*** of DNA component B.

L16 ANSWER 22 OF 33 MEDLINE
ACCESSION NUMBER: 84297241 MEDLINE
DOCUMENT NUMBER: 84297241 PubMed ID: 6089116
TITLE: The histone H5 gene is flanked by S1 hypersensitive structures.
AUTHOR: Ruiz-Carrillo A
SOURCE: NUCLEIC ACIDS RESEARCH, ***(1984 Aug 24)*** 12 (16)
6473-92.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198410
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19841012

AB The potential of the cloned histone H5 gene to form altered DNA structures
has been examined by S1 ***nuclease*** digestion of supercoiled recombinant plasmids containing up to 8.8 kbp of chicken DNa. The three main nicking sites map at the upstream and downstream sequences flanking
the structural gene. The ***cleavage*** sites share sequence homology, strand specificity, and do not seem to be ***single*** - ***stranded*** . The sequence of the S1-sensitive sites does not suggest that the fragments can adopt any of the known DNA secondary structures.

L16 ANSWER 23 OF 33 MEDLINE
ACCESSION NUMBER: 84069794 MEDLINE
DOCUMENT NUMBER: 84069794 PubMed ID: 6316268
TITLE: A site and strand specific nuclease activity with analogies to topoisomerase I frames the rRNA gene of Tetrahymena.
AUTHOR: Gocke E; Bonven B J; Westergaard O
SOURCE: NUCLEIC ACIDS RESEARCH, ***(1983 Nov 25)*** 11 (22)
7661-78.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198401
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19840126

AB Exposure of macronuclear chromatin from Tetrahymena thermophila to sodium dodecyl sulfate causes an endogenous ***nuclease*** to ***cleave*** the extra-chromosomal rDNA at specific sites. All cuts are ***single*** - ***strand*** ***cleavages*** specific to the non-coding strand. Three ***cleavages*** map in the central non-transcribed spacer of the palindromic molecule at positions -1000, -600 and -150 bp with respect to the transcription initiation point. A fourth site is located close to the transcription termination point, while no ***cleavage*** is observed in the coding region. The position of each ***cleavage*** is in the immediate neighbourhood of DNAse I hypersensitive sites. Additionally, certain DNA sequence motifs are repeated in the region around the ***cleavages*** . Upon ***cleavage*** induction a protein becomes attached to the rDNA. Our results indicate covalent binding to the generated 3' end, in analogy to the aborted reaction of topoisomerase I.

L16 ANSWER 24 OF 33 MEDLINE
ACCESSION NUMBER: 81117278 MEDLINE
DOCUMENT NUMBER: 81117278 PubMed ID: 6257691
TITLE: Functional inactivation of lac alpha-peptide mRNA by a factor that purifies that Escherichia coli RNase III.
AUTHOR: Shen V; Cynamon M; Daugherty B; Kung H; Schlessinger D
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, ***(1981 Feb 25)*** 256 (4) 1896-902.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198104
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19810421

AB Using RNA-directed synthesis of the alpha-peptide of beta-galactosidase as an assay, a factor was purified that inactivated further function of the mRNA. In the presence of Ca2+ ions to inhibit most ***nuclease*** activity, inactivation of mRNA occurred during incubation with ribosomes or with a 1 M KCl wash of ribosomes. The inactivation activity required Mg2+ ions, and purified as a single factor which did not bind to DEAE-cellulose, but bound reversibly to phosphocellulose. The factor eluted from Sephadex G-150 with an apparent molecular weight of about 43,000. Purified 700-fold, it showed no detectable exonuclease activity, and little or no ***cleavage*** of a variety of ***single*** - ***stranded*** substrates, including full length lac operon mRNA; but repurified inactivated mRNA was still inactive for protein synthesis. The factor did not inhibit poly(U)-directed polyphenylalanine synthesis. When proteins isolated from the ribosomal wash were individually tested, highly purified RNase III, which purifies in the same way and has the same size, also inactivated lac mRNA. The ribosomal wash from an RNase III- strain showed little if any activity compared to that from an isogenic RNase III+ strain. The possibility of a site-specific inactivating ***cleavage*** of mRNA by RNase III at or near the 5' end is considered.

L16 ANSWER 25 OF 33 MEDLINE
ACCESSION NUMBER: 76095015 MEDLINE
DOCUMENT NUMBER: 76095015 PubMed ID: 942717
TITLE: Site of cleavage of superhelical phiX174 replicative form DNA by the single strand-specific Neurospora crassa endonuclease.
AUTHOR: Bartok K; Denhardt D T
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, ***(1976 Jan 25)*** 251 (2) 530-5.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197604
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19970203
Entered Medline: 19760402

AB Experiments with the Neurospora crassa ***single*** ***strand*** -specific endonuclease have provided evidence for the existence of regions of partially ***single*** - ***stranded*** character in covalently closed superhelical replicative form DNA of phiX174. The ***nuclease*** converts the superhelical molecules to either singly hit relaxed circular or doubly hit linear molecules. We show that the initial ***cleavage*** of phiX174 superhelical DNA is a "nick" bounded by a 5'-phosphate and a 3'-hydroxyl; no nucleotides are excised as evidenced by the ability of T4-polynucleotide ligase to reform the phosphodiester bond. The nick can be found in either strand of the double-stranded DNA and is either randomly distributed or at least can be found at any one of many possible locations in the genome. Thus, the regions in phiX174 superhelical molecules that are sensitive to the N. crassa ***nuclease*** do not occur at highly specific sites in the genome.

L16 ANSWER 26 OF 33 MEDLINE
ACCESSION NUMBER: 75207480 MEDLINE
DOCUMENT NUMBER: 75207480 PubMed ID: 167982
TITLE: Nucleic acid ***hybridization*** using DNA covalently coupled to cellulose.
AUTHOR: Noyes B E; Stark G R
SOURCE: CELL, ***(1975 Jul)*** 5 (3) 301-10.
Journal code: CQ4; 0413066. ISSN: 0092-8674.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197511
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19751107

AB We describe a method for linking RNA and DNA covalently to finely divided cellulose through a diazotized aryl amine, which reacts primarily with guanine and uracil (thymine) residues of ***single*** ***strands*** . The high efficiency of coupling and high capacity of the cellulose for nucleic acid make possible a product with as much as 67 mug of nucleic acid per mg of cellulose. The product is especially suitable for ***hybridization*** experiments where very low backgrounds are important, and it is stable in 99% formamide at 80 degrees C so that ***hybridized*** nucleic acid can be recovered easily. Full length linear Simian Virus 40 (SV40) DNA, produced by ***cleavage*** of SV40(I) DNA with S1 ***nuclease*** , can be coupled to diazo cellulose with an efficiency of 80-90%, and is effective in ***hybridization*** experiments with SV40 DNA, complementary RNA synthesized in vitro from SV40(I) DNA with E. coli RNA polymerase, and the SV40-specific fraction of total RNA from SV40-infected and transformed cells. In these experiments an excess of cellulose-bound DNA was used, and the efficiency of ***hybridization*** was about 90% when ribonuclease treatment of the hybrids was omitted.

L16 ANSWER 27 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:575379 HCAPLUS
DOCUMENT NUMBER: 119:175379
TITLE: ***Hybridization*** assay using branched nucleic acid probes
INVENTOR(S): Hogan, James John; Arnold, Lyle John, Jr.; Nelson, Norman Charles; Bezverkov, Robert
PATENT ASSIGNEE(S): Gen-Probe Inc., USA
SOURCE: Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 552931	A1	19930728	EP 1993-300377	19930120 <--

EP 552931 B1 20000524
R: CH, DE, FR, GB, IT, LI, SE
US 5424413 A 19950613 US 1992-940652 19920904
WO 9315102 A1 19930805 WO 1993-US486 19930121 <--
W: AU, CA, JP, KR
AU 9335866 A1 19930901 AU 1993-35866 19930121 <--
AU 665062 B2 19951214
JP 07503139 T2 19950406 JP 1993-513301 19930121
US 5451503 A 19950919 US 1994-255553 19940607
PRIORITY APPLN. INFO.: US 1992-827021 19920122
WO 1993-US486 19930121

AB The title ***hybridization*** probes contg. .gtoreq.2 target nucleic acid-specific regions and arm regions which are complementary to the arm regions of another probes, which arms do not ***hybridize*** to each other in the absence of the target nucleic acid. In the presence of the targets, the probes will anneal to the targets and to the complementary arms of other probes to form a branched structure. The amt. of target nucleic acid can be detd. by detecting the formation of the resultant structure after the ***hybridization*** of the arm regions which involves ***cleavage*** by resolvase or S1 ***nuclease*** or restriction endonuclease, DNA footprint anal., gel electrophoresis, or use and chem. modification of intercalating agent (e.g. acridinium ester). The arm region of the probes optionally contains an extending ***single*** - ***stranded*** region for the formation of .gtoreq.1 secondary arms, contains a duplex region to serve as primer for DNA polymerase or as promoter for an RNA polymerase, contains a DNA/RNA duplex susceptible to RNase H ***cleavage*** , or contains a site adjacent to the duplex nucleic acid which is ***cleavable*** by Fe-EDTA or phenanthroline. With the method, a target rRNA (of Neisseria gonorrhoeae) was clearly distinguished from the potentially cross-reacting target nucleic acid with 2 mismatches (of Neisseria meningitidis).

L16 ANSWER 28 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:507791 HCAPLUS
DOCUMENT NUMBER: 115:107791
TITLE: ***Hybridization*** probe-based apparatus and method for gene mutation detection
INVENTOR(S): Nagai, Keiichi; Tokita, Jiro
PATENT ASSIGNEE(S): Hitachi, Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 6 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 03043098	A2	19910225	JP 1989-175465	19890710 <--
US 5194372	A	19930316	US 1990-548798	19900706 <--

PRIORITY APPLN. INFO.: JP 1989-175465 19890710
JP 1989-224419 19890901

AB A method for detecting genetic mutation involves: treating a test genetic substance with fluorescent or fluorescence substrate-using enzyme-labeled ***single*** - ***stranded*** nucleic acid probe, treating with enzymes (S1 ***nuclease*** , RNase) to ***cleave*** noncomplementary mismatches, sepg. the ***cleaved*** segments from the intact complementary segments by electrophoresis, and scanning the labeled segments with laser radiation. An app. consisting of the probe, an electrophoresis device, and the laser scanner for the detection also is claimed. The segments are subjected to electrophoresis on polyacrylamide gel or agarose gel. Using the method (app.), 6 base-deficient .beta.-galactosidase gene-contg. DNA (M13) was tested.

L16 ANSWER 29 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:435251 HCAPLUS
DOCUMENT NUMBER: 111:35251
TITLE: Sequence-targeted cleavage of single- and double-stranded DNA by oligothymidylates covalently linked to 1,10-phenanthroline
AUTHOR(S): Francois, Jean Christophe; Saison-Behmoaras, Tula; Chassignol, Marcel; Nguyen Thanh Thuong; Helene, Claude
CORPORATE SOURCE: Lab. Biophys., CNRS, Paris, 75005, Fr.
SOURCE: J. Biol. Chem. (***1989***), 264(10), 5891-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ***nuclease*** activity of the 1,10-phenanthroline(OP)-Cu complex was targeted to a specific sequence by attachment of the ligand to the 5'- or 3'-end of octathymidylate [(dT)8]. An acridine deriv. was also attached to the other end of the (dT)8-OP conjugate. The duplex formed by the (dT)8 with its complementary sequence was stabilized by intercalation of the acridine deriv. The reaction conditions adopted involved ***hybridization*** of the (dT)8-OP conjugate to a 27-nucleotide-long (27-mer) DNA fragment contg. a (dA)8 sequence prior to addn. of Cu2+ and 3-mercaptopropionic acid (MPA). The reaction induced by MPA led to a very localized ***cleavage*** of the 27-mer sequence. Control expts. indicated that the ***cleavage*** reaction could be obtained only if (dT)8-OP, Cu2+, and MPA were present. Max. degrdn. was obtained when Cu2+ was added after MPA in a buffered soln. contg. the 27-mer (dA)8 and the (dT)8-OP conjugate. At high NaCl concn. or in the presence of spermine, ***cleavage*** of the ***single*** - ***stranded*** 27-mer fragment occurred on both sides of the target sequence. This was ascribed to the formation of a triple helix involving 2 (dT)8-OP strands that adopted an antiparallel orientation with respect to each other. When a 27-mer duplex was used as a substrate, ***cleavage*** sites were obsd. on both strands. The location of the ***cleavage*** sites led to the conclusion that (dT)8 was bound to the (dA)8.cntdot.(dT)8 sequence in a parallel orientation with respect to the (dA)8-contg. strand. This result reflected the ability of thymine to form 2 H-bonds with an adenosine already engaged in a Watson-Crick base pair. The results showed that it is possible to design DNA-binding oligodeoxynucleotides that can selectively recognize and ***cleave*** polypurine-polypyrimidine sequences in double-stranded DNA.

L16 ANSWER 30 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:88778 HCAPLUS
DOCUMENT NUMBER: 108:88778
TITLE: Characterization of a pro-.alpha.2(I) collagen gene mutation by nuclease S1 mapping
AUTHOR(S): Pihlajaniemi, Taina; Myers, Jeanne C.
CORPORATE SOURCE: Dep. Med. Biochem., Univ. Oulu, Oulu, 90220, Finland
SOURCE: Methods Enzymol. (***1987***), 145(Struct. Contract. Proteins, Pt. E), 213-22
CODEN: MENZAU; ISSN: 0076-6879
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The mol. defect in a patient with a moderately severe form of osteogenesis imperfecta (OI) was characterized by ***nuclease*** S1 mapping. ***Single*** - ***stranded*** 5' and 3' end-labeled cDNA probes coding for 80% of the carboxyl-propeptide of the human pro-.alpha.2(I) collagen chain were ***hybridized*** to poly(A)+ RNA isolated from cultured skin fibroblasts. ***Nuclease*** S1 digestion and denaturing polyacrylamide gel electrophoresis allowed the identification of a homozygous mutation in the patient's mRNA and a heterozygous pattern in the RNAs from the consanguinous parents. Subsequent genomic cloning and sequencing of the OI patient's DNA revealed a four-base-pair frameshift deletion changing the last 33 amino acids of the carboxyl-propeptide. The mutation prevented incorporation of pro-.alpha.2(I) chains into the normal type I procollagen heterotrimer [.alpha.[1(I)2.alpha.2(I)]], resulting in secretion of only pro-a1(I) homotrimers. This paper describes the isolation of poly(A)+ RNA, prepn. of 32P-labeled cDNA probes, and the conditions used for ***hybridizationjand*** ***nuclease*** S1 digestion, which permitted complete ***cleavage*** of the DNA at the small region of mismatch.

L16 ANSWER 31 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1987:472104 HCAPLUS
DOCUMENT NUMBER: 107:72104

TITLE: Methods and materials for obtaining microbial expression of polypeptides including bovine prolactin
INVENTOR(S): Souza, Lawrence M.
PATENT ASSIGNEE(S): AMGEN, USA
SOURCE: U.S., 16 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4666839	A	19870519	US 1982-445986	19821201 <--

AB A method for selective modification of double-stranded DNAs to facilitate their storage and incorporation into expression vectors comprises (1) obtaining a single-stranded DNA copy of a double-stranded DNA of interest; (2) ***hybridizing*** a primer to a selected portion of the single-stranded DNA; (3) extending the primer in the presence of nucleotides triphosphates and DNA polymerase, reconstructing the original double-stranded DNA sequence except for the sequence 3' to the site of ***hybridization*** ; and (4) treating the extension product to remove the single-stranded region. Cloning and expression of bovine prolactin and chicken growth hormone cDNAs in Escherichia coli were achieved using the above method by ***hybridizing*** primers to single-stranded M13 clones contg. the cDNAs at a location downstream of the leader sequences, followed by deletion of the leader sequences and addn. of start codons (ATG) via synthetic linkers.

L16 ANSWER 32 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:517099 HCAPLUS
DOCUMENT NUMBER: 99:117099
TITLE: Association of an S1 nuclease-sensitive structure with short direct repeats 5' of Drosophila heat shock genes
AUTHOR(S): Mace, Hilary A. F.; Pelham, Hugh R. B.; Travers, Andrew A.
CORPORATE SOURCE: Lab. Mol. Biol., Med. Res. Counc. Cent., Cambridge, CB2 2QH, UK
SOURCE: Nature (London) (***1983***), 304(5926), 555-7
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The 5' flanking DNA sequences for the D. melanogaster heat-shock gene hsp70 were examd. for the presence of S1 ***nuclease*** [37288-25-8]-sensitive sites. Apparently, a sequence necessary to define the S1 ***cleavage*** site occurs between -130 and -138, and a sequence upstream of -138 influences the proportion of plasmid mols. contg. the S1 ***cleavage*** site. The hsp70 gene and adjacent sequences were contained in 3 recombinant plasmids. Plasmid pHI1 contained the entire hsp70 coding region and 1140 base pairs (bp) of the 5' flanking sequence, whereas plasmid pHT.DELTA.5'-186 contained only 186 bp of the 5' flanking sequence. The 3rd plasmid (pH2) contained the SV40 virus origin region juxtaposed to the 5' side of the hsp70 gene. ***Nuclease*** treatment of these plasmids resulted in rapid conversion of their supercoiled DNA to nicked, circular DNA which, in turn, was slowly converted to the linear form. Southern ***hybridization*** anal. of DNA restriction fragments indicated S1 ***cleavage*** with each plasmid at -140 of the hsp70 gene. More precise mapping indicated that the major termini generated by S1 ***nuclease*** centered at -124 in pHT.DELTA.5'-186. With pHI1, however, the termini centered at approx. -145. Anal. of 5' flanking sequence deletion derivs. of pHT.DELTA.5'-186 indicated that when the hsp70 sequence was extended to position -138, the S1 ***nuclease*** site was still present, but the proportion of plasmid DNA ***cleaved*** was reduced .apprx.5-fold. A model is proposed in which the termini pattern is generated from a structure in which the hexanucleotide (CT)3 sequence at -137 to -132 in the upper strand pairs with the hexanucleotide (GA)3 at -122 to -117 in the lower strand to form 2 ***single*** - ***stranded*** loops. Similar S1 ***nuclease*** -sensitive sites are assocd. with short direct repeats of DNA in the 5' flanking regions of other D. melanogaster heat-shock genes.

L16 ANSWER 33 OF 33 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:196367 HCAPLUS
DOCUMENT NUMBER: 98:196367
TITLE: Chymosin
INVENTOR(S): Carey, Norman Henry; Doel, Michael Terence; Harris, Timothy John Roy; Lowe, Peter Anthony; Emtage, John Spencer
PATENT ASSIGNEE(S): Celltech Ltd., UK
SOURCE: Eur. Pat. Appl., 58 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 68691	A2	19830105	EP 1982-303035	19820611 <--
EP 68691	A3	19830810		
R: DE, FR, GB, IT, NL, SE				
AU 8284810	A1	19821223	AU 1982-84810	19820611 <--
AU 555175	B2	19860918		
GB 2100737	A1	19830106	GB 1982-17096	19820611 <--
GB 2100737	B2	19850123		
DK 8202714	A	19821218	DK 1982-2714	19820616 <--
JP 58009687	A2	19830120	JP 1982-104672	19820617 <--
JP 07095881	A2	19950411	JP 1994-95496	19940411
PRIORITY APPLN. INFO.:			GB 1981-18688	19810617
			GB 1981-33998	19811111
			GB 1981-36185	19811201
			GB 1982-3907	19820210

AB A process is described for the prodn. of chymosin [9001-98-3] by the ***cleavage*** of methionine-chymosin [85713-24-2], methionine-prochymosin [85713-26-4], or preprochymosin [85713-29-7] expressed by a host organism transformed with cloned cDNAs that encode these products. Thus, mRNA isolated from the abomasal mucosa of unweaned calves was used to prep. ***single*** - ***stranded*** cDNA with avian viral reverse transcriptase [9068-38-6] and the primer oligo(dT)12-18 or the sequence-specific primers 5'-d(GTTCATCATGTT)-3' [85632-81-1]. ***Single*** - ***stranded*** cDNA was made double-stranded by incubation with reverse transcriptase; the hairpin at one end was removed with S1 ***nuclease*** [37288-25-8], and raggedness at the ends was repaired with DNA polymerase I [9012-90-2] Klenow fragment. To ensure retention of nucleotide sequences at the end of the cDNA coding region, an alternative method that involved treatment ***single*** - ***stranded*** cDNA extension with terminal transferase [9027-67-2] was employed. Double-stranded cDNA was cloned into the PstI [81295-32-1] site of plasmid pAT153 by the homopolymer tailing method and the annealed DNA was used to transform Escherichia coli. Recombinant clones were screened by colony ***hybridization*** , and a recombinant clone designated 118 was constructed by the joining DNA from 2 clones contg. portions of a prochymosin [9059-50-1]-encoding sequence. The preprochymosin sequence was deduced from the cDNA sequence, and the prochymosin sequence differed from the published sequence in 2 residues. Expression vectors were constructed for E. coli and Saccharomyces cerevisiae, and these included vectors in which an ATG initiation codon was attached directly to chymosin- or prochymosin-specifying cDNA, as well as vectors for the expression of preprochymosin. E. coli Transformed with plasmid pCT70, which contains cDNA that encodes methionine-prochymosin, contained methionine-prochymosin as .apprx.5% of the cell protein. Methods were developed for the purifn. of methionine-prochymosin and for the recovery of chymosin from prochymosin (40% recovery with respect

to

clotting activity) by acid treatment. A micro method for the assay of chymosin activity was also developed.

09/660924
AH#8

1. Document ID: US 20010014450 A1

L7: Entry 1 of 16 File: PGPB Aug 16, 2001

PGPUB-DOCUMENT-NUMBER: 20010014450
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010014450 A1

TITLE: Detection of differences in nucleic acids

PUBLICATION-DATE: August 16, 2001
US-CL-CURRENT: 435/6; 435/91.2, 435/91.5

APPL-NO: 09/ 732279
DATE FILED: December 7, 2000

RELATED-US-APPL-DATA:

RLAN	RLFD	RLPC	RLKC	RLAC
09732279	Dec 7, 2000	ABANDONED	A1	US
09370919	Aug 9, 1999	GRANTED		US
09370919	Aug 9, 1999			US
08771623	Dec 20, 1996			US
6013439	Mar 6, 1996			US
60012929	Dec 22, 1995			US
60009289				

IN: Lishanski, Alla, Kurn, Nurith, Ullman, Edwin F.

AB: A method is disclosed for detecting the presence of a difference between two related nucleic acid sequences. In the method a complex is formed comprising both strands of each sequence. Each member of at least one pair of non-complementary strands within the complex have labels. The association of the labels as part of the complex is determined as an indication of the presence of a difference between the two related sequences. The complex generally comprises a Holliday junction. In one aspect a medium suspected of containing said two related nucleic acid sequences is treated to provide partial duplexes having non-complementary tailed portions at one end. The double stranded portions of the partial duplexes are identical except for said difference. One of the strands of one of the partial duplexes is complementary to one of the strands of the other of the partial duplexes and the other of the strands of one of the partial duplexes is complementary to the other of the strands of the other of the partial duplexes. The medium is subjected to conditions that permit the binding of the tailed portions of the partial duplexes to each other. If there is a difference in the related nucleic acid sequences, a stable complex is formed comprising a Holliday junction. If no difference exists, the complex dissociates into duplexes. A determination is made whether the stable complex is formed, the presence thereof indicating the presence of the related nucleic acid sequences. The method has application in detecting the presence of a mutation in a target sequence or in detecting the target sequence itself.

L7: Entry 1 of 16 File: PGPB Aug 16, 2001

DOCUMENT-IDENTIFIER: US 20010014450 A1
TITLE: Detection of differences in nucleic acids

BSTX:
[0011] Various methods for mutation detection have been developed in the recent years based on amplification technology. The detection of sequence alterations is based on one of the following principles: allele-specific hybridization, chemical modification of mismatched bases with subsequent strand cleavage, nuclease cleavage at mismatches, recognition of mismatches by specific DNA binding proteins, changes in electrophoretic mobility of mismatched duplexes in gradients of denaturing agents, conformation-induced changes in electrophoretic mobility of single-stranded DNA sometimes combined with conformation-specific nuclease cleavage. Some of these methods are too laborious and time-consuming and many depend on the nature of base alteration.

2. Document ID: US 6232104 B1

L7: Entry 2 of 16 File: USPT May 15, 2001

US-PAT-NO: 6232104
DOCUMENT-IDENTIFIER: US 6232104 B1
TITLE: Detection of differences in nucleic acids by inhibition of spontaneous DNA branch migration
DATE-ISSUED: May 15, 2001

US-CL-CURRENT: 435/91.2; 435/6, 536/23.1, 536/24.2, 536/24.33

APPL-NO: 9/ 376097
DATE FILED: August 17, 1999

IN: Lishanski; Alla, Taylor; Marc, Kurn; Nurith

AB: A method is disclosed for detecting the presence of a difference between two related nucleic acid sequences. In the method a complex is formed comprising both strands of each sequence. Each member of at least one pair of non-complementary strands within the complex have labels. The association of the labels as part of the complex is determined as an indication of the presence of a difference between the two related sequences. The complex generally comprises a Holliday junction. In one aspect a medium suspected of containing said two related nucleic acid sequences is treated to provide partial duplexes having non-complementary tailed portions

at one end. The double stranded portions of the partial duplexes are identical except for said
difference. One of the strands of one of the partial duplexes is complementary to one of the
strands of the other of the partial duplexes and the other of the strands of one of the partial
duplexes is complementary to the other of the strands of the other of the partial duplexes. The
medium is subjected to conditions that permit the binding of the tailed portions of the partial
duplexes to each other. If there is a difference in the related nucleic acid sequences, a
stable complex is formed comprising a Holliday junction. If no difference exists, the complex
dissociates into duplexes. A determination is made whether the stable complex is formed, the
presence thereof indicating the presence of the related nucleic acid sequences. The method has
application in detecting the presence of a mutation in a target sequence or in detecting the
target sequence itself. Also provided is an alternative primer scheme which allows for the
reduction of background signal due to mis-priming during amplification of the nucleic acid
sequences in the detection method described herein.

L7: Entry 2 of 16 File: USPT May 15, 2001

DOCUMENT-IDENTIFIER: US 6232104 B1
TITLE: Detection of differences in nucleic acids by inhibition of spontaneous DNA branch migration

BSPR:
Various methods for mutation detection have been developed in the recent years based on
amplification technology. The detection of sequence alterations is based on one of the following
principles: allele-specific hybridization, chemical modification of mismatched bases with subsequent
strand cleavage, nuclease cleavage at mismatches, recognition of mismatches by specific DNA binding
proteins, changes in electrophoretic mobility of mismatched duplexes in gradients of denaturing
agents, conformation-induced changes in electrophoretic mobility of single-stranded DNA sometimes
combined with conformation-specific nuclease cleavage. Some of these methods are too laborious and
time-consuming and many depend on the nature of base alteration.

3. Document ID: US 6210950 B1

L7: Entry 3 of 16 File: USPT Apr 3, 2001

US-PAT-NO: 6210950
DOCUMENT-IDENTIFIER: US 6210950 B1
TITLE: Methods for diagnosing, preventing, and treating developmental disorders due to a combination
of genetic and environmental factors
DATE-ISSUED: April 3, 2001

US-CL-CURRENT: 435/252.3; 435/183, 435/320.1, 536/23.1, 536/24.31, 536/24.33

APPL-NO: 9/ 318448

DATE FILED: May 25, 1999

IN: Johnson; William G., Stenroos; Edward Scott

AB: The present invention discloses a novel method for identifying an individual who may
be susceptible to develop a developmental disorder. In one particular example, an individual is
identified who is genetically susceptible to becoming schizophrenic. In addition, the present
invention discloses a novel method for identifying individuals who are genetically susceptible
to have offspring with a developmental disorder. Methods of diagnosing, preventing and treating
developmental disorders such as schizophrenia are also provided.

L7: Entry 3 of 16 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210950 B1
TITLE: Methods for diagnosing, preventing, and treating developmental disorders due to a combination
of genetic and environmental factors

DEPR:
There are many methods currently known in the art to identify variant/mutant DNA, all of which may
be used in the present invention (see e.g., internet address
http://www.ich.bpmf.ac.uk/cmgs/mutdet.htm). Such methods include but in no way are limited to direct
sequencing, array sequencing, matrix-assisted laser desorption/ionization time-of-flight mass
spectrometry (Malditof) [Fitzgerald et al., Ann. Rev. Biophy. Biomol. Struct. 24:117-140 (1995)],
Polymerase Chain Reaction "PCR", reverse-transcriptase Polymerase Chain Reaction "RT-PCR", RNAase
protection assays, Array quantitation e.g., as commercially provided by Affymetrix, Ligase Chain
Reaction or Ligase Amplification Reaction (LCR or LAR), Self-Sustained Synthetic Reaction
(3SR/NASBA), Restriction Fragment Length Polymorphism (RFLP),Cycling Probe Reaction (CPR),
Single-Strand Conformation Polymorphism (SSCP), heteroduplex analysis, hybridization mismatch using
nucleases (e.g., cleavase), Southern, Northerns, Westerns, South Westerns, ASOs, Molecular beacons,
footprinting, and Fluorescent In Situ Hybridization (FISH). Some of these methods are briefly
described below.

4. Document ID: US 6200781 B1

L7: Entry 4 of 16 File: USPT Mar 13, 2001

US-PAT-NO: 6200781
DOCUMENT-IDENTIFIER: US 6200781 B1
TITLE: Apparatus, system and method for automated execution and analysis of biological and chemical
reactions
DATE-ISSUED: March 13, 2001

US-CL-CURRENT: 435/91.1; 422/131, 435/287.2, 435/6

APPL-NO: 9/ 339865

DATE FILED: June 25, 1999

IN: Tal; Michael, Liran; Yoram, Koren; Zvi

AB: An apparatus for controlling the temperature of at least one liquid reaction
mixture, the apparatus including (a) at least one reaction vessel having open proximal and
distal ends, the at least one reaction vessel including a gas permeable, liquid retaining,
barrier positioned at a proximal portion thereof; (b) a pump being in fluid communication with
the proximal end of the at least one reaction vessel through the barrier, for generating
negative or positive pressure within the at least one reaction vessel, for translocating the at
least one liquid reaction mixture through the distal end into and out of the at least one
reaction vessel; and (c) a temperature controller being in thermal communication with the at
least one reaction vessel for controlling the temperature of the at least one liquid reaction
mixture when maintained within the at least one reaction vessel.

L7: Entry 4 of 16

File: USPT

Mar 13, 2001

DOCUMENT-IDENTIFIER: US 6200781 B1
TITLE: Apparatus, system and method for automated execution and analysis of biological and chemical
reactions

DEPR:
In addition, methods of post reaction analysis of nucleic acid products include, but are not limited
to, allele specific oligonucleotide (ASO) hybridization; reverse-ASO; denaturing/temperature
gradient gel electrophoresis (D/TGGE); single-strand conformation polymorphism (SSCP); heteroduplex
analysis; restriction fragment length polymorphism (RFLP); nuclease protection assays; chemical
cleavage and other, less frequently used, methods. Each of these reactions can be performed by a
dedicated analyzer subsequent to the termination of the reaction simply by ejecting via the pump the
content of the vessels or samples therefrom into a multititer plate, treating the samples as
required and analyzing the results, obviating the need to open each vessel independently.

PARENT-CASE:
This application claims the benefit of U.S. Provisional Application No. 60/012,752, filed Mar. 4,
1996.

IN: Monforte; Joseph Albert, Shaler; Thomas Andrew, Tan; Yuping, Becker; Christopher
Hank

AB: This invention relates to methods for screening nucleic acids for mutations by
analyzing nonrandomly fragmented nucleic acids using mass spectrometric techniques and to
procedures for improving mass resolution and mass accuracy of these methods of detecting

L7: Entry 5 of 16

File: USPT

Apr 18, 2000

DOCUMENT-IDENTIFIER: US 6051378 A
TITLE: Methods of screening nucleic acids using mass spectrometry

DEPR:
FIG. 7 shows how different portions of the single-stranded target nucleic acid are hybridized to the
oligonucleotide probes. Following hybridization, any regions of the target nucleic acid that remain
single-stranded are cleaved using a single-strand-specific endo/exonuclease, such as S1 Nuclease,
Mung bean nuclease, or RNase A. The size of the single-stranded region can be as small as a single
phosphodiester bridge, i.e. the phosphodiester bond across from a nick. S1 nuclease is capable of
cleaving across from nicks. The end products are double-stranded hybrids comprised of two equal
length strands: one strand is a member of the set of nonrandom length fragments derived from the
target nucleic acid and the other strand is a member of the set of fragmenting probes, wherein said
NLFs are hybridized to said fragmenting probes. Either these double-stranded hybrids or isolated
single-stranded nonrandom length fragments derived from said target nucleic acid can be used for
MALDI-TOF mass spectrometric analysis. Preferably, the analysis of the single-stranded nonrandom
length fragments derived from said target nucleic acid provides a simpler mass spectrum. It should
be noted that when the complementary strands are a mixed DNA/RNA hybrid there will be a significant
mass difference between the two strands in all cases, making each strand more easily resolvable in
the mass spectrum.

5. Document ID: US 6051378 A

L7: Entry 5 of 16

File: USPT

Apr 18, 2000

US-PAT-NO: 6051378
DOCUMENT-IDENTIFIER: US 6051378 A
TITLE: Methods of screening nucleic acids using mass spectrometry
DATE-ISSUED: April 18, 2000

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2, 536/23.1, 536/24.1, 536/24.33

APPL-NO: 8/ 811505
DATE FILED: March 4, 1997

6. Document ID: US 6013439 A

L7: Entry 6 of 16

File: USPT

Jan 11, 2000

US-PAT-NO: 6013439
DOCUMENT-IDENTIFIER: US 6013439 A
TITLE: Detection of differences in nucleic acids
DATE-ISSUED: January 11, 2000

US-CL-CURRENT: 435/6; 536/25.32

APPL-NO: 8/ 771623
DATE FILED: December 20, 1996

PARENT-CASE:
This application claims benefit of provisional application No. 60/012,929 filed Mar. 6, 1996, this
application claims benefit of provisional application No. 60/009,289 filed Dec. 22, 1995.

IN: Lishanski; Alla, Kurn; Nurith, Ullman; Edwin F.

AB: A method is disclosed for detecting the presence of a difference between two related
nucleic acid sequences. In the method a complex is formed comprising both strands of each
sequence. Each member of at least one pair of non-complementary strands within the complex have
labels. The association of the labels as part of the complex is determined as an indication of
the presence of a difference between the two related sequences. The complex generally comprises
a Holliday junction. In one aspect a medium suspected of containing said two related nucleic
acid sequences is treated to provide partial duplexes having non-complementary tailed portions
at one end. The double stranded portions of the partial duplexes are identical except for said
difference. One of the strands of one of the partial duplexes is complementary to one of the
strands of the other of the partial duplexes and the other of the strands of one of the partial
duplexes is complementary to the other of the strands of the other of the partial duplexes. The
medium is subjected to conditions that permit the binding of the tailed portions of the partial
duplexes to each other. If there is a difference in the related nucleic acid sequences, a
stable complex is formed comprising a Holliday junction. If no difference exists, the complex
dissociates into duplexes. A determination is made whether the stable complex is formed, the
presence thereof indicating the presence of the related nucleic acid sequences. The method has
application in detecting the presence of a mutation in a target sequence or in detecting the
target sequence itself.

L7: Entry 6 of 16
File: USPT
Jan 11, 2000

DOCUMENT-IDENTIFIER: US 6013439 A
TITLE: Detection of differences in nucleic acids

BSPR:
Various methods for mutation detection have been developed in the recent years based on
amplification technology. The detection of sequence alterations is based on one of the following
principles: allele-specific hybridization, chemical modification of mismatched bases with subsequent
strand cleavage, nuclease cleavage at mismatches, recognition of mismatches by specific DNA binding
proteins, changes in electrophoretic mobility of mismatched duplexes in gradients of denaturing
agents, conformation-induced changes in electrophoretic mobility of single-stranded DNA sometimes
combined with conformation-specific nuclease cleavage. Some of these methods are too laborious and
time-consuming and many depend on the nature of base alteration.

7. Document ID: US 5928872 A

L7: Entry 7 of 16
File: USPT
Jul 27, 1999

US-PAT-NO: 5928872
DOCUMENT-IDENTIFIER: US 5928872 A
TITLE: Subtractive hybridization with covalently binding homology
DATE-ISSUED: July 27, 1999

US-CL-CURRENT: 435/6; 435/91.2

APPL-NO: 8/ 927859
DATE FILED: September 11, 1997

PARENT-CASE:
The present application is a continuation-in-part of application Ser. No. 08/854,400 filed May 12,
1997.

IN: Lin; Shi-Lung, Ying; Shao-Yao

AB: Excess amount of modified subtracter DNA from control cells is generated by
carboxylating the base structures of its certain nucleotides with chemical agents in order to
introduce covalent affinity between the modified subtracter and a non-modified tester DNA.
Hybridization of the control subtracter and the experimental tester DNA is performed with a
heat-melting and then cool-reassociation technique. While the desired different (heterologous)
sequences remain in the form of hydrogen-binding, common (homologous) sequences of the
hybridized DNA are covalently bonded to each other. Since the covalent bonding of the common
sequences can not be broken during a polymerase chain reaction, resulting in no amplification
of the common sequences but great amplification of the desired different sequences. The desired
DNA sequences present after such covalent homologue subtraction and selective amplification
represent those DNA sequences which only exist in the tester but not in the subtracter DNA
library.

L7: Entry 7 of 16
File: USPT
Jul 27, 1999

DOCUMENT-IDENTIFIER: US 5928872 A
TITLE: Subtractive hybridization with covalently binding homology

DEPR:
The present invention is directed to an improved subtractive hybridization method, called nucleotide
analog-containing DNA subtraction assay (NDSA), for screening different sequences between two cDNA
or genomic DNA libraries. This method is primarily designed for quickly isolating different
expression genes (either up- or down-regulated), easily detecting large genomic
deletions/insertions, and precisely searching chromosome-specific loci. The preferred version of the
present invention is based on: the nucleotide analog-incorporated subtracter hybridization with
non-modified tester DNA, the abasic-nick/gap generation in common sequences by a nucleotide
analog-removing enzyme, and the abasic-nick/gap cleavage by a single-strand-specific nuclease. In

conjunction with an adaptor-ligation and a specific PCR amplification, a very small amount of DNA
library can be used as an initial sample for this method.

8. Document ID: US 5871927 A

L7: Entry 8 of 16

File: USPT

Feb 16, 1999

US-PAT-NO: 5871927
DOCUMENT-IDENTIFIER: US 5871927 A
TITLE: Nucleotide analog-containing hybrid subtraction with sequentially enzymatic digestion
DATE-ISSUED: February 16, 1999

US-CL-CURRENT: 435/6; 435/91.2

APPL-NO: 8/ 854400
DATE FILED: May 12, 1997

IN: Lin; Shi-Lung, Ying; Shao-Yao

AB: The present invention provides a method for fast, simple, and reliable isolation of
desired different sequences from two DNA libraries. Excess amount of nucleotide
analog-containing DNA subtracter from control cells is generated by incorporating nucleotide
analog with a template-dependent extension reaction to introduce susceptible-sites for
subsequent enzymatic digestion. Hybridization of the control subtracter and experimental DNA is
performed with a heat-melting and then cool-reassociation technique. The hybridized DNAs are
subtracted with nucleotide analog-removing enzyme first, resulting in nicking or gapping all
nucleotide analog-containing hybrid duplexes which are further digested by
single-strand-specific nuclease. Desired DNA sequences from the experimental cells, but not the
control ones stay intact throughout the digestion procedure and can be selectively amplified at
the end. This technique is designed for the subtractive hybridization of different sequences
between two DNA libraries from distinct cell sources and will allow more efficient isolations
in experiments on cancer formation, development of gene therapy, and understanding of
pathological status and developmental regulation.

L7: Entry 8 of 16

File: USPT

Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5871927 A
TITLE: Nucleotide analog-containing hybrid subtraction with sequentially enzymatic digestion

DEPR:
The present invention is directed to an improved subtractive hybridization method, called nucleotide
analog-containing DNA subtraction assay (NDSA), for screening different sequences between two cDNA
or genomic DNA libraries. This method is primarily designed for quickly isolating different
expression genes (either up- or down-regulated), easily detecting large genomic
deletions/insertions, and precisely searching chromosome-specific loci. The preferred version of the
present invention is based on: the nucleotide analog-incorporated subtracter hybridization with
non-modified tester DNA, the abasic-nick/gap generation in common sequences by a nucleotide
analog-removing enzyme, and the abasic-nick/gap cleavage by a single-strand-specific nuclease. In
conjunction with an adaptor-ligation and a specific PCR amplification, a very small amount of DNA
library can be used as an initial sample for this method.

9. Document ID: US 5843650 A

L7: Entry 9 of 16

File: USPT

Dec 1, 1998

US-PAT-NO: 5843650
DOCUMENT-IDENTIFIER: US 5843650 A
TITLE: Nucleic acid detection and amplification by chemical linkage of oligonucleotides
DATE-ISSUED: December 1, 1998

US-CL-CURRENT: 435/6; 435/4, 435/5, 435/91.1, 435/91.2, 435/91.3, 536/23.1, 536/24.3, 536/24.31,
536/24.32, 536/24.33

APPL-NO: 8/ 431527
DATE FILED: May 1, 1995

IN: Segev; David

AB: The present invention and kits are directed to a method of amplifying and detecting
single or double-stranded target nucleic acid molecules in a test sample. Amplification is
accomplished through the use of a minimum of two oligonucleotide probe complement pairs,
wherein members oligonucleotide probes from both pair of oligonucleotide probe complement pairs
form a minimum of two oligonucleotide probe pairs, at least one of which is complementary to a
given portion of a target nucleic acid sequence which act as template. One of the
oligonucleotide probes of each oligonucleotide probe pair have an additional protecting
sequence which is not complementary to the target sequence. These additional protecting
sequences are preferably complementary to each other. Chemical functionality groups attached to
the oligonucleotide probes covalently combine the probes to form a joined oligonucleotide
product. The joined oligonucleotide product is formed without the use of enzymes. The
reactivity of the chemical functionality groups on each probe is target dependent. The chemical
functionality group on each probe is prevented from reacting with other chemical functionality
groups on other probes unless the probes are properly hybridized to the target molecule. The
chemical functionality groups are covalently attached to the oligonucleotide probes in such a
way that they are sheltered or protected from the chemical functionality groups of other probes
while the probes are in solution. Only when the oligonucleotide probes of an oligonucleotide
probe pair are hybridized to the target sequence are the chemical functionality groups on the

probes brought into close enough proximity to form a covalent bond and join the probes to form
a joined oligonucleotide product. Once formed, the joined oligonucleotide product is denatured
from the target nucleic acid molecule thereby doubling the amount of target sequences
originally present in the sample. The process is repeated a desired number of times to produce
detectable amounts of joined oligonucleotide products.

L7: Entry 9 of 16

File: USPT

Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843650 A
TITLE: Nucleic acid detection and amplification by chemical linkage of oligonucleotides

BSPR:
Other methods which have been used to determine the presence of alterations in known DNA sequences
include allele specific oligonucleotide (ASO) hybridization; reverse-ASO; restriction site
generating PCR (RG-PCR); denaturing/temperature gradient gel electrophoresis (D/TGGE); single-strand
conformation polymorphism (SSCP); heteroduplex analysis; restriction fragment length polymorphism
(RFLP); PCR restriction fragment length polymorphism (PCR-RFLP); nuclease protection assays;
chemical cleavage and other, less frequently used, methods.

DEPR:
As far as the enzyme-based methods such as allele specific oligonucleotide probe (ASO)
hybridization; reverse-ASO; restriction site generating PCR (RG-PCR); denaturing/temperature
gradient gel electrophoresis (D/TGGE); single-strand conformation polymorphism (SSCP); heteroduplex
analysis; restriction fragment length polymorphism (RFLP); PCR restriction fragment length
polymorphism (PCR-RFLP); nuclease protection assays; chemical cleavage and other, less frequently
used, methods are concerned, the method of the present invention acts as an enzyme-free system for
selective amplification of target nucleic acid sequences and enjoys a number of advantages:

10. Document ID: US 5770370 A

L7: Entry 10 of 16

File: USPT

Jun 23, 1998

US-PAT-NO: 5770370
DOCUMENT-IDENTIFIER: US 5770370 A
TITLE: Nuclease protection assays
DATE-ISSUED: June 23, 1998

US-CL-CURRENT: 435/6; 435/196, 435/199, 435/91.1, 435/91.2, 436/501, 536/23.1, 536/24.1, 536/24.3,
536/24.31, 536/24.32 , 536/24.33

APPL-NO: 8/ 665104
DATE FILED: June 14, 1996

IN: Kumar; Rajan

AB: The invention provides nuclease protection assay comprising: (A) attaching a nucleic
acid probe comprising a first nucleotide sequence to a solid surface area; (B) contacting the
nucleic acid probe with a nucleic acid template under conditions that promote hybridization
between complementary polynucleotides, forming a probe-template complex if the template
includes a segment that is complementary to the probe; (C) contacting the probe-template
complex with a nuclease effective to selectively cleave the nucleotide bonds of (1) the first
nucleotide sequence when the first nucleotide sequence is single stranded or (2) mismatched
regions of the first nucleotide sequence when the first nucleotide sequence is in duplex
nucleic acid; and (D) detecting the presence of duplex nucleic acids formed by the probe and
template nucleic acids by detecting the presence of the first nucleotide sequence.

L7: Entry 10 of 16

File: USPT

Jun 23, 1998

DOCUMENT-IDENTIFIER: US 5770370 A
TITLE: Nuclease protection assays

ABPL:
The invention provides nuclease protection assay comprising: (A) attaching a nucleic acid probe
comprising a first nucleotide sequence to a solid surface area; (B) contacting the nucleic acid
probe with a nucleic acid template under conditions that promote hybridization between complementary
polynucleotides, forming a probe-template complex if the template includes a segment that is
complementary to the probe; (C) contacting the probe-template complex with a nuclease effective to
selectively cleave the nucleotide bonds of (1) the first nucleotide sequence when the first
nucleotide sequence is single stranded or (2) mismatched regions of the first nucleotide sequence
when the first nucleotide sequence is in duplex nucleic acid; and (D) detecting the presence of
duplex nucleic acids formed by the probe and template nucleic acids by detecting the presence of the
first nucleotide sequence.

BSPR:
The invention provides nuclease protection assay comprising: (A) attaching a nucleic acid probe
comprising a first nucleotide sequence to a solid surface area; (B) contacting the nucleic acid
probe with a nucleic acid template under conditions that promote hybridization between complementary
polynucleotides, forming a probe-template complex if the template includes a segment that is
complementary to the probe; (C) contacting the probe-template complex with a nuclease effective to
selectively cleave the nucleotide bonds of (1) the first nucleotide sequence when the first
nucleotide sequence is single stranded or (2) mismatched regions of the first nucleotide sequence
when the first nucleotide sequence is in duplex nucleic acid; and (D) detecting the presence of
duplex nucleic acids formed by the probe and template nucleic acids by detecting the presence of the
first nucleotide sequence. In one embodiment, the attaching step occurs prior to the first
contacting step. In one embodiment, the attaching comprises synthesizing the nucleic acid probe on
the solid surface area. In one embodiment, the probe is attached to the solid surface area
subsequent to hybridization between the probe and a template DNA.

Preferably, the solid surface area
comprises plastic, glass, cellulose or a cellulose derivative, nylon or other synthetic membranous
material, or ceramic. In one embodiment, the solid surface area is a microparticle, which is
preferably paramagnetic.

BSPR:
The assay of the invention, together with all its preferred and alternate embodiments, can be used
as the basis for a method for the sequencing of a sample polynucleotide, the method comprising: (A)
preparing an array having a multitude of distinct nucleic acid probes, each of known nucleotide
sequence, each comprising a first polynucleotide sequence, and each immobilized at a separate,
identifiable solid surface area in the array, with each immobilized nucleic acid probe comprising a
label attached to the nucleic acid probe, which label is releasable from the nucleic acid probe when
any nucleotide bond of the first polynucleotide sequence of the attached nucleic acid probe is
cleaved; (B) contacting the array with the sample polynucleotide or fragments thereof under
conditions that promote hybridization between complementary nucleic acids; (C) contacting the solid
surface areas of the array with a nuclease effective to selectively cleave the nucleotide bonds of
(1) a first polynucleotide sequences when such a first polynucleotide sequence is single stranded or
(2) mismatched regions of a first polynucleotide sequence when such a first polynucleotide sequence
is in duplex nucleic acid; (D) removing released label from the solid surface areas; and (E)
detecting any label remaining on the solid surface areas and identifying the nucleic acid probe
immobilized at the solid surface areas where label is detected. The sequencing method can further
comprise: (F) compiling the sub-sequences of the sample polynucleotide identified through their
protection from nuclease digestion, which protection results in the detection of label at the solid
surface area of a complementary first polynucleotide sequence; and (G) aligning the detected
sequences by identifying sequence overlaps between the compiled sub-sequences. The steps F and G are
done manually or by computer. In this method, preferably, the first polynucleotide sequences of the
nucleic acid probes comprise at least about 8-mers. Preferably, the first nucleotide sequences are
8-mers and the array comprises first polynucleotide sequences for each of the 65,536 possible such
sequences or multiple arrays are processed which together comprise first polynucleotide sequences
for each of the 65,536 possible such sequences.

11. Document ID: US 5753439 A

L7: Entry 11 of 16
File: USPT
May 19, 1998

US-PAT-NO: 5753439
DOCUMENT-IDENTIFIER: US 5753439 A
TITLE: Nucleic acid detection methods
DATE-ISSUED: May 19, 1998

US-CL-CURRENT: 435/6; 435/5, 435/91.2, 536/24.3, 536/24.32, 536/24.33

APPL-NO: 8/446102

DATE FILED: May 19, 1995

IN: Smith; Cassandra L., Yaar; Ron, Szafranski; Przemyslaw, Cantor; Charles R.

AB: The invention relates to methods for rapidly determining the sequence and/or length
a target sequence. The target sequence may be a series of known or unknown repeat sequences
which are hybridized to an array of probes. The hybridized array is digested with a
single-strand nuclease and free 3'-hydroxyl groups extended with a nucleic acid polymerase.
Nuclease cleaved heteroduplexes can be easily distinguish from nuclease uncleaved
heteroduplexes by differential labeling. Probes and target can be differentially labeled with
detectable labels. Matched target can be detected by cleaving resulting loops from the
hybridized target and creating free 3-hydroxyl groups. These groups are recognized and extended
by polymerases added into the reaction system which also adds or releases one label into
solution. Analysis of the resulting products using either solid phase or solution. These
methods can be used to detect characteristic nucleic acid sequences, to determine target
sequence and to screen for genetic defects and disorders. Assays can be conducted on solid
surfaces allowing for multiple reactions to be conducted in parallel and, if desired,
automated.

L7: Entry 11 of 16
File: USPT
May 19, 1998

DOCUMENT-IDENTIFIER: US 5753439 A
TITLE: Nucleic acid detection methods

DEPR:
This hybridized array, either fixed or free in solution, is digested with a single-strand specific
nuclease to cleave single stranded regions such as heteroduplexes and terminal extensions. Nucleases
suitable for digestion of hybridized probes include those nuclease which preferentially cleave
single-stranded nucleic acids. Preferred nucleases include the endonucleases such as S1 nuclease,
mung-bean nuclease, ribonuclease A and ribonuclease T1. Nucleic acids or probes which generate
terminal single strands can be digested with exonucleases such as the T4 and T7 phage nucleases.
When desired, treatment with excess nuclease can be directed to produce double-stranded cleavage by
extending the nick to a gap and thereby creating a single-stranded region on the opposite strand.
Such double-stranded cuts can be useful in procedures where probes are fragmented.

12. Document ID: US 5710028 A

L7: Entry 12 of 16
File: USPT
Jan 20, 1998

US-PAT-NO: 5710028

DOCUMENT-IDENTIFIER: US 5710028 A
TITLE: Method of quick screening and identification of specific DNA sequences by single nucleotide
primer extension and kits therefor
DATE-ISSUED: January 20, 1998

US-CL-CURRENT: 435/91.1; 435/6, 536/24.33

APPL-NO: 8/ 317432
DATE FILED: October 4, 1994

PARENT-CASE:
This is a continuation in part of U.S. patent application Ser. No. 08/084,505, filed Jul. 1, 1993,
now abandoned which is a continuation in part of U.S. patent application Ser. No. 07/919,872, filed
Jul. 27, 1992, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
IL 102382 July 2, 1992

IN: Eyal; Nurit, Navot; Nir

AB: A method of simultaneous determination of the identity of nucleotide bases at
specific positions in nucleic acids of interest, which includes (a) treating a sample
containing the nucleic acids of interest to obtain unpaired nucleotide bases spanning the
specific positions, if the nucleic acids are not already single stranded; (b) contacting the
unpaired nucleotide bases with combinations of various marked oligonucleotide primers each for
hybridizing with a stretch of nucleotide bases present in each nucleic acid of interest
immediately adjacent the nucleotide base to be identified, so as to form a duplex between the
primer and the nucleic acid of interest such that the nucleotide base to be identified is the
first unpaired base in the template immediately 5' of the nucleotide base annealed with the
3'-end of the primer in the duplex; (c) contacting the duplex with the reagent which includes
an aqueous carrier, and at least one primer extension unit, the primer extension unit including
an extension moiety a separation moiety and a detection moiety, with the extension moiety for
specifically halting a nucleic acid template dependent, primer extension reaction, in a manner
which is strictly dependent on the identity of the unpaired nucleotide base of the template
immediately adjacent to, and 3' of, the 3'-end of the primer, with the separation moiety
permitting the affinity separation of the primer extension unit from unincorporated, or
non-extended, primers, and with the detection moiety enabling the direct or indirect detection
of the presence of a primer extension unit the contacting taking place under conditions
permitting the base pairing of the complementary extension moiety of the primer extension unit
present in the reagent with the nucleotide base to be identified and the occurrence of a
template dependent, primer extension reaction to incorporate the extension moiety of the primer
extension unit at the 3'-end of the primer, resulting in the extension of the primer by a
single unit; (d) removing the non-extended marked primer; (e) determining the presence of a
nucleotide alteration; and (f) determining the identity of the extended primers, and therefore
the kind of alterations and the complete genotype of the examined nucleic acid, by hybridizing
the extended primers to complementary oligonucleotides adhered to a test surface.

L7: Entry 12 of 16 File: USPT Jan 20, 1998

DOCUMENT-IDENTIFIER: US 5710028 A
TITLE: Method of quick screening and identification of specific DNA sequences by single nucleotide
primer extension and kits therefor

BSPR:
Other methods which have been used to determine the presence of alterations in known DNA sequences
include ligase chain reaction (LCR); allele specific oligonucleotide (ASO) hybridization;
reverse-ASO; restrictive site generating PCR (RG-PCR); denaturing/temperature gradient gel
electrophoresis (D/TGGE); single strand conformation polymorphism (SSCP); heteroduplex analysis;
restriction fragment length polymorphism (RFLP); PCR restriction fragment length polymorphism
(PCR-RFLP); nuclease protection assays; chemical cleavage and other, less frequently used, methods.

13. Document ID: US 5582972 A

L7: Entry 13 of 16 File: USPT Dec 10, 1996

US-PAT-NO: 5582972
DOCUMENT-IDENTIFIER: US 5582972 A
TITLE: Antisense oligonucleotides to the RAS gene
DATE-ISSUED: December 10, 1996

US-CL-CURRENT: 435/6; 435/91.3, 536/23.1, 536/24.1, 536/24.5

APPL-NO: 7/ 990303
DATE FILED: December 14, 1992

PARENT-CASE:
This is a continuation-in-part application of application Ser. No. 07/715,196, filed Jun. 14, 1991,
now abandoned.

IN: Lima; Walter, Monia; Brett, Freier; Susan, Ecker; David

AB: Methods are provided for preparing antisense oligonucleotides which take advantage
of RNA secondary and tertiary structure and for preparing antisense oligonucleotides which
specifically hybridize to regions of RNA secondary and tertiary structure by comparing the
affinity of the oligonucleotide for a structured RNA target to the affinity of the
oligonucleotide for a length-matched oligonucleotide complement, and selecting an
oligonucleotide having an affinity for the structured target which is not less than one
thousandth of its affinity for the length-matched oligonucleotide complement. Oligonucleotides
are also disclosed which are specifically hybridizable with regions of H-ras RNA having

secondary and tertiary structure.

L7: Entry 13 of 16

File: USPT

Dec 10, 1996

DOCUMENT-IDENTIFIER: US 5582972 A
TITLE: Antisense oligonucleotides to the RAS gene

DEPR:
For the three oligonucleotides targeted to the loop, thermodynamic effect of the hairpin depends on target site. These effects cannot be explained by simple base-pairing thermodynamics; rather, loop structure is responsible. For all three antisense oligonucleotides, the target site is single stranded as demonstrated by cleavage with single strand-specific nucleases, and no base pairs should have to be broken for hybridization to occur. It appears the thermodynamic cost of binding to residues 43-52 is similar to that of binding to a stem region. Binding to residues 33-42, on the other hand, has a small negative cost; it is slightly easier to bind to the loop structure than the free single strand.

14. Document ID: US 5359051 A

L7: Entry 14 of 16

File: USPT

Oct 25, 1994

US-PAT-NO: 5359051
DOCUMENT-IDENTIFIER: US 5359051 A
TITLE: Compounds useful in the synthesis of nucleic acids capable of cleaning RNA
DATE-ISSUED: October 25, 1994

US-CL-CURRENT: 536/26.7; 536/24.5, 536/25.34

APPL-NO: 7/ 846556
DATE FILED: March 5, 1992

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of application Ser. No. US91/00243, filed Jan. 11, 1991, which is a continuation-in-part of application Ser. No. 463,358, filed Jan. 11, 1990 and application Ser. No. 566,977, filed Aug. 13, 1990 both now abandoned. These applications are assigned to the assignee of this invention. The entire disclosure of each is incorporated herein by reference.

IN: Cook; Phillip D., Guinosso; Charles J., Bruice; Thomas

AB: Compositions and methods for modulating the activity of RNA are disclosed. In accordance with preferred embodiments, antisense compositions are prepared comprising targeting and reactive portions. In preferred embodiments, the reactive portions comprise one or two imidazole functionalities conjugated to the targeting oligonucleotide via linkers with and without intervening intercalating moieties and act through phosphorodiester hydrolytic bond cleavage. Therapeutics, diagnostics and research methods are also disclosed. Synthetic nucleosides and nucleoside fragments are also provided which are useful for elaboration of oligonucleotides for such purposes.

L7: Entry 14 of 16

File: USPT

Oct 25, 1994

DOCUMENT-IDENTIFIER: US 5359051 A
TITLE: Compounds useful in the synthesis of nucleic acids capable of cleaning RNA

BSPR:
Certain compositions useful for effecting the cleavage of an RNA molecule in accordance with this invention generally comprise a sugar modified oligonucleotide containing a targeting sequence which is specifically hybridizable with a preselected nucleotide sequence of single stranded or double stranded DNA or RNA molecule and which is nuclease resistant. The sequence is synthesized, typically through solid state synthesis of known methodology, to be complementary to or at least to be specifically hybridizable with the preselected nucleotide sequence of the RNA or DNA. Nucleic acid synthesizers are commercially available and their use is generally understood by persons of ordinary skill in the art as being effective in generating nearly any oligonucleotide of reasonable length which may be desired.

15. Document ID: US 4870009 A

L7: Entry 15 of 16

File: USPT

Sep 26, 1989

US-PAT-NO: 4870009
DOCUMENT-IDENTIFIER: US 4870009 A
TITLE: Method of obtaining gene product through the generation of transgenic animals
DATE-ISSUED: September 26, 1989

US-CL-CURRENT: 435/69.4; 800/25, 800/5

APPL-NO: 6/ 561644
DATE FILED: December 15, 1983

PARENT-CASE:
This application is a continuation-in-part of U.S. application Ser. No. 443,071, filed Nov. 22, 1982 in the names of R. D. Palmiter and R. L. Brinster and now abandoned.

IN: Evans; Ronald M., Palmiter; Richard D., Brinster; Ralph L.

AB: Mammalian genes that encode hormones are cloned and linked to strong promoter DNA sequences. The linked sequences are inserted in plasmids for amplification in prokaryotic cells, and multiple copies of the linked sequences are excised therefrom. Linked sequences are subsequently microinjected into fertilized eggs and the fertilized eggs are implanted into pseudo-pregnent females of the same species. As a result, transgenic animals are born having the linked sequences incorporated into their genomes and expressing the

gene-encoded hormone.
Because multiple copies of the linked sequences are frequently inserted and because production
of the hormone is not limited to certain organs, as is the case with most endogenous hormones,
the transgenic animals produce substantial amounts of the hormone. Hormone can be harvested
from the living animal (and from its hormone-producing progeny) by extracting fluid, such as
blood serum or ascites fluid, on a regular basis.

L7: Entry 15 of 16

File: USPT

Sep 26, 1989

DOCUMENT-IDENTIFIER: US 4870009 A
TITLE: Method of obtaining gene product through the generation of transgenic animals

DEPR:
If all processing signals in the fusion gene are correctly being recognized, a fusion mRNA (63
nucleotides larger than bona fide rat GH mRNA) would be generated. Denaturing gel eletrophoresis and
RNA Northern blot analysis of the liver RNA from MGH-21 showed that its size is indistinguishable
from authentic mouse and rat pituitary GH mRNA. Liver RNA from a control mouse shows no GH-reactive
sequences. Because the GH DNA probe used for the RNA blot analysis recognizes both rat and mouse GH
mRNAs, it was necessary to establish that the hybridizing species in the liver is actually the
product of the fusion gene and not mouse GH mRNA due to an unexpected activation of the endogenous
mouse GH gene. The use of an Xho I linker for the construction of the fusion gene generates a
sequence that will be uniquely present in MGH mRNA. Thus, the Xho I site of pMGH was labeled using
Y.sup.32 P-ATP and polynucleotide kinase followed by cleavage with Sst I. This 217 nucleotide
fragment was gel purified, denatured, and used as hybridization probe in a single-strand specific
nuclease protection assay. Hybridization to MGH mRNA should generate a 74 base nuclease-resistant
fragment while mouse GH mRNA or metallothionein mRNA will be unable to protect the kinased end and
should therefore be negative. The results of this analysis showed that the predicted 74 base
fragment is present in liver RNA of mouse MGH-21, but not in normal mouse pituitary RNA, control
mouse liver RNA or in the liver RNA of MGH-3, an animal negative for growth. Thus, it appears that
transcription is initiating properly at the MT-I promoter and continuing through the putative
termination site of the GH gene, that the four GH intervening sequences are being properly spliced
and that the MGH mRNA is polyadenylated.

16. Document ID: US 5770370 A

L7: Entry 16 of 16

File: EPAB

Jun 23, 1998

PUB-NO: US005770370A
DOCUMENT-IDENTIFIER: US 5770370 A
TITLE: Nuclease protection assays

PUBN-DATE: June 23, 1998

INT-CL (IPC): C12Q 1/68; C12P 19/34; C07H 21/02; C07H 21/04
EUR-CL (EPC): C12Q001/68; C12Q001/68

APPL-NO: US66510496
APPL-DATE: June 14, 1996

PRIORITY-DATA: US66510496A (June 14, 1996)

IN: KUMAR, RAJAN

AB: CHG DATE=19990617 STATUS=O>The invention provides nuclease protection assay
comprising: (A) attaching a nucleic acid probe comprising a first nucleotide sequence to a
solid surface area; (B) contacting the nucleic acid probe with a nucleic acid template under
conditions that promote hybridization between complementary polynucleotides, forming a
probe-template complex if the template includes a segment that is complementary to the probe;
(C) contacting the probe-template complex with a nuclease effective to selectively cleave the
nucleotide bonds of (1) the first nucleotide sequence when the first nucleotide sequence is
single stranded or (2) mismatched regions of the first nucleotide sequence when the first
nucleotide sequence is in duplex nucleic acid; and (D) detecting the presence of duplex nucleic
acids formed by the probe and template nucleic acids by detecting the presence of the first
nucleotide sequence.

L7: Entry 16 of 16

File: EPAB

Jun 23, 1998

DOCUMENT-IDENTIFIER: US 5770370 A
TITLE: Nuclease protection assays

FPAR:
CHG DATE=19990617 STATUS=O>The invention provides nuclease protection assay comprising: (A)
attaching a nucleic acid probe comprising a first nucleotide sequence to a solid surface area; (B)
contacting the nucleic acid probe with a nucleic acid template under conditions that promote
hybridization between complementary polynucleotides, forming a probe-template complex if the
template includes a segment that is complementary to the probe; (C) contacting the probe-template
complex with a nuclease effective to selectively cleave the nucleotide bonds of (1) the first
nucleotide sequence when the first nucleotide sequence is single stranded or (2) mismatched regions
of the first nucleotide sequence when the first nucleotide sequence is in duplex nucleic acid; and
(D) detecting the presence of duplex nucleic acids formed by the probe and template nucleic acids by
detecting the presence of the first nucleotide sequence.